



Review

Sjögren's Syndrome: Immunological Response Underlying the Disease

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Abstract. Sjögren's syndrome is a chronic autoimmune disorder characterized primarily by the discomforts of dry eyes and dry mouth due to the progressive loss of exocrine gland function. Development of a number of animal models to study Sjögren's syndrome, especially the NOD mouse and its congenic partner strains, has permitted a systematic analysis of immunological and non-immunological factors that influence predisposition for development of the autoimmune response. These data are reviewed here.

Key words: NOD mouse; autoimmune exocrinopathy; cytokine knock-out mice; autoantibody.

Introduction

Sjögren's syndrome is defined as a chronic inflammatory autoimmune disorder primarily affecting the lacrimal and salivary exocrine glands. However, the disorder has also been associated with numerous extraglandular manifestations affecting such diverse tissues as the cardiovascular, central nervous, and renal systems¹⁹. Diagnostic criteria include oral and/or ocular dryness, focal lymphocytic infiltrates in the exocrine tissues, and the presence of various autoantibodies in patient sera, particularly against the nuclear factors SS-A/Ro and SS-B/La. Due to inconsistencies in the various diagnostic scales, evaluation of disease prevalence worldwide is somewhat difficult and further complicated by the chronic and inconsistent nature of the symptoms in patients. Recently, the clinical features and epidemiology of Sjögren's syndrome have been adequately reviewed (see FOX et al.¹⁹, FOX and SPEIGHT¹⁸); therefore, this review will restrict its dis-

cussion to immunological features of Sjögren's syndrome.

Typically, Sjögren's syndrome is diagnosed in its later stages, when focal lymphocytic infiltration and glandular destruction have already occurred. For this reason, the initial and early events in the pathogenesis of the disease remain largely unknown. Conversely, the persisting immunological response in the context of the minor salivary glands has been studied to a much greater depth. This is, in part, a reflection of the relative accessibility of the minor salivary glands in obtaining tissue biopsies. This has allowed for the assessment of cytokine activity, as well as the categorization of infiltrating leukocyte populations in the glands of patients exhibiting an ongoing disease process. During the past couple of decades, investigators have established several inbred mouse strains as applicable animal models for the further study of Sjögren's syndrome pathogenesis. Among others, the MRL/lpr, NFS/sld and the NOD and NOD.B10.H-2^b mouse strains have emerged

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as the most popular animal models for Sjögren's syndrome based on immune infiltration of the exocrine tissues, cytokine profiles, and serology^{24-26, 47}. Additionally, the NOD and NOD.B10.H-2^b mice exhibit an immune-dependent loss of secretory function in both the lacrimal and salivary glands. Assuming the appropriateness of these animal models, our understanding of both the initiation and development of Sjögren's syndrome has advanced markedly, from a reasonable appreciation of the later stages of the disease, to the determination of numerous additional autoantigens and, more importantly, insights into the initiating events in the disease.

Etiology of Sjögren's Syndrome

Like other autoimmune disorders, Sjögren's syndrome appears to be a multifactorial disease, with its onset dependent on both intrinsic and extrinsic parameters. From a genetic standpoint, there appears to be an association with certain MHC haplotypes, especially DR3 and DQ2 in patient populations⁴, and genomic analysis in animal models has already uncovered regions on chromosomes 1, 3, 4, 10, and 18 associated with susceptibility^{11, 41}. Since these chromosomal regions are polygenic, it is impossible at this time to state definitively whether the regulatory elements encoded in these loci are immunological in nature. However, glandular remodeling occurs in the submandibular glands of NOD-*scid* mice in the absence of an adaptive immune response⁴¹ and this strongly suggests that non-immunological regulators exist. In the NOD mouse model, chromosome 1 contains a susceptibility locus which appears important in the development of sialitis¹⁷ as well as the focal infiltration of the lacrimal glands¹¹. A summary of the genetic loci including some of the potential genes of interest are included in Table 1. With the application of emerging technologies, such as microchip array systems, to study gene expression in

the affected exocrine tissues, we will hopefully gain a clearer insight into the non-immunologic aspects of Sjögren's syndrome pathogenesis.

More than any other body of evidence, the studies in the NOD-*scid* mouse have promoted the idea that the exocrinopathy may not be entirely dependent on the immune infiltrate. This animal model exhibits gross morphological restructuring³¹ and elevated levels of apoptosis in the salivary and lacrimal epithelial cells despite the absence of glandular lymphocytic foci³¹. Furthermore, while proapoptotic proteins such as caspase-3 and Bax are elevated in acinar and ductal tissues¹⁴ along with bcl-2 (an anti-apoptotic factor) in NOD and NOD-*scid* mice, the infiltrating lymphocytic populations found in the NOD exocrine glands appear to upregulate bcl-2 only¹⁸, while maintaining normal expression levels of caspase-3 and Bax. This suggests that the infiltrates may reside within the exocrine glands in a protected state. The presence of constitutively expressed FasL and induced Fas expression on the salivary epithelial cell surfaces at progressive stages of disease, along with the increase in TUNEL staining indicative of excessive programmed cell death¹⁶, encourages the belief that an underlying disturbance in glandular homeostasis could potentially facilitate or even promote immunopathological activity.

Non-immunological factors influencing the development of Sjögren's syndrome also include hormonal regulation, as suggested by the sexual dimorphism (9 : 1 in favor of females over males) found in the patient population. This has been supported by numerous lacrimal studies in models such as the MRL and NZB/NZW mouse strains, where androgens tend to exert a protective influence for dacryoadenitis⁵¹. Interestingly, the NOD mouse presents a reversal of androgen influence, where the presence of androgens correlates to increased lacrimal destruction^{27, 55}. In the case of the influence of the sex hormones on disease pathogenesis, it is worth emphasizing that, while endocrine regulation may not itself be directly immunological in nature, studies have

Table 1. Susceptibility loci mapped in mouse models of Sjögren's syndrome^{a, b}

Chromosome	Mouse model	cM length	Genes of interest
1	NOD/MRL/lpr	40/3.9	<i>IL-1R, caspase-8, caspase-10, CTLA-4, pgs-2</i> ^c
3	NOD	0.35	<i>IL-2</i>
4	MRL/lpr (female only)	16.9	<i>Faf1, IFNA1, IFNB1, Jun, Lck</i>
10	MRL/lpr	19	<i>MIF, ITGB2</i>
18	MRL/lpr	8	<i>FGF1, GRL</i>

^a From ENCINAS, et. al.¹³.

^b From NISHIHARA, et. al.⁴¹.

^c These genes are all potential candidates within the NOD-derived genome segment.

demonstrated the abilities of estrogens and androgens to regulate the immune response^{3, 22}.

Environmental factors have been studied in association with events leading to localized immune activation and to immune dysregulation and disease onset. Viral-induced onset of disease tends to receive a significant amount of attention and, along these lines, several viral pathogens have been identified as potential etiologic agents for Sjögren's syndrome. Several members of the gamma herpesvirus family, most notably Epstein-Barr virus (EBV)^{16, 54} and human herpesvirus type 6 (HHV-6)¹⁷, have been detected at a slightly higher frequency in Sjögren's syndrome patients. Sicca symptoms have also been associated with elevated serum antibody titres against hepatitis C virus (HCV)². Despite the extensive studies on viral etiologic agents leading to Sjögren's syndrome, no single virus has been conclusively identified as a direct causative agent and, quite possibly, multiple chronic insults may act in concert to trigger the necessary immune activation leading to autoimmune exocrinopathy.

Cytokine Profiles in Sjögren's Syndrome

Cytokine expression in the salivary glands of Sjögren's syndrome patients plus that observed in various mouse models suggests a pro-inflammatory profile. Cytokine mapping in the salivary and lacrimal glands has relied largely on the measurement of stable mRNA levels via reverse transcriptase-polymerase chain reaction. Evaluation of patient populations reveal the expression of IL-1 β , IL-2, IL-6, IL-10, IL-12, IL-18, TNF- α , TGF- β and IFN- γ in the minor labial glands. Similarly, studies in the mouse models report the expression of IL-1 β , IL-2, IL-6, IL-7, IL-10, IL-12, TNF- α , TGF- β , iNOS and IFN- γ in the submandibular and lacrimal glands^{10, 27}. In addition, IL-5 is noted in the murine lacrimal gland^{40, 45}. Rarely, IL-4 can be detected but is generally considered absent in the infiltrated glands. More recent studies in both human and mouse salivary glands have indicated that this comprehensive pro-inflammatory pattern of cytokine expression can be detected in the salivary tissues of healthy donors as well as non-autoimmune mouse strains at levels similar to disease-state values^{32, 60}. It would appear, therefore, that rather than being a defining hallmark of the disease process, these cytokine mRNA expression profiles are more a reflection of tissue specific micro-regulation of the immune responses favoring a pro-inflammatory response in these glands in the context of localized immune activation. In support of this, Fox, et. al.¹⁹ showed

that acinar and ductal epithelial cells express a wide variety of cytokine mRNA, including IL-2, IL-6, IL-10, TNF- α , IFN- γ and TGF- β , while other reports indicate that IFN- γ can induce upregulation of IL-1 β , IL-6 and TNF- α , along with HLA-DR and ICAM-1 in HSG cells^{23, 59}. In contrast to mRNA levels, the protein expression of the infiltrating lymphocytes and epithelial cells in murine salivary glands, as measured by immunocytochemistry, is markedly different between NOD versus Balb/c control strains. In the case of the pro-inflammatory cytokines, significantly greater quantities can be detected in association with the Sjögren's syndrome-like disease state⁶⁰. In essence, this accentuates an extremely important point, i.e. while mRNA levels in a cell may reflect gene activation, gene expression and gene product activity are regulated on many levels beyond transcription by cellular manipulation of translation rates, post-translational modifications, and even product sequestration. Therefore, mRNA expression levels may not necessarily equate with protein expression. In fact, this appears to be the case in terms of glandular cytokine expression.

Ongoing studies using cytokine knockout mice are revealing interesting new insights regarding the autoimmune pathogenesis. Although the detection of specific cytokine expressions in target tissues may reveal an underlying role for that cytokine, this approach operates under the assumption that the entire pathological course of events occurs in the target tissues. Studies employing cytokine-deficient congenic animals emphasize an essential consideration that critical aspects of the pathogenic processes in Sjögren's syndrome occur outside the environment of the exocrine glands.

Just as there are limitations to looking at cytokine expression levels in the target tissues, it can be misleading to define the importance of a cytokine based on changes in disease pathogenesis in its absence or overexpression. Certainly, it has been shown many times over that the activity of a cytokine is not simply controlled by its presence or absence, but also by the level and timing of its expression^{39, 42}. Additionally, many cytokines, for instance IL-4 and IL-13, have overlapping effects, such that the elimination of an individual cytokine from an *in vivo* system may not exhibit as dramatic an effect, despite the distinct role of the factor in the normal disease process⁶². This can be seen, to a certain extent, in the differential effects, of cytokine versus cytokine receptor deletions⁸. Regardless, gene-knockout models eliminating either cytokines or cell populations provide unique new perspectives into the questions or relevance with regard to these immune components.

Analysis of the NOD.IL-4⁻ mouse exemplifies the highly significant contribution of studies introducing cytokine deficiencies into a disease-susceptible mouse to map cytokine functions. IL-4 is rarely if ever detected in salivary or lacrimal gland homogenates in studies of mouse models, or in labial gland biopsies of patients, yet this cytokine is absolutely critical in driving the suppression of secretory function in the autoimmune exocrinopathy in the context of the NOD mouse. The NOD.IL-4⁻ mouse develops massive focal lymphocytic infiltrates in the exocrine glands despite maintaining normal secretory capacity throughout its life (unpublished data). Further elucidation of the composition of the infiltrating lymphocytes reveals a decreased percentage of B cells compared with the NOD parental strain. This finding supports accumulating evidence that the secretory suppression in the lacrimal and salivary glands is a direct result of autoantibodies acting on the fluid-secreting acinar cells¹⁴.

Another cytokine that has received increasing attention is IL-10. IL-10 expression increases notably over time in the course of disease progression and, to make this factor even more interesting, there is a viral homologue secreted by EBV³⁸, a virus associated with Sjögren's syndrome manifestation. Recent studies in which a transgene construct introducing IL-10 under the control of the salivary amylase promoter to induce exocrinopathy show that the induction of IL-10 expression in the salivary glands can lead to focal infiltration and secretory suppression in the C57BL/6 genetic background⁴⁹. However, in the context of the Sjögren's syndrome-like pathology, introduction of the IL-10-deficiency mutation into the NOD mouse contradicts the findings derived from IL-10 overexpression. The lack of IL-10 apparently has little effect, as these animals develop normal, if not exacerbated, focal infiltration and disease progression. This should not necessarily be interpreted as a contradiction, but rather as a prime example of the importance of timing of expression as opposed to strict cytokine presence in directing an immune response. Taken together, the studies on overexpression and deficiency of IL-10 suggest that this cytokine would appear to be capable of contributing to the underlying pathology but is not, in and of itself, absolutely necessary for Sjögren's-like disease progression. These data begin to establish parallels to the other autoimmune diseases such as diabetes, where more extensive studies into the role of IL-10 in disease onset in the NOD model imply that IL-10 acts in a distinctly time-dependent fashion, such that it promotes and even accelerates diabetes initiation when expressed constitutively in the pancreatic tissues,

but serves to ameliorate pancreatitis when administered later in the course of the disease^{5, 6, 43, 58}.

Characterization of Infiltrating Lymphocytes

As cytokine profiles suggest, the focal lymphocytic infiltrates found in the salivary and lacrimal glands of Sjögren's patients as well as of mouse models are comprised predominantly of CD4⁺ αβ T cells. In the human disease, lymphocytes comprise approximately 80% of the focal infiltrates¹⁵. Plasma B cells present in the lesions produce mostly IgM and IgG³⁷ and have a greater than normal tendency to mutate into lymphomas⁵³, one of the more serious and certainly lethal consequences of Sjögren's syndrome. The salivary infiltrates in the NOD mouse are comprised of approximately 45% CD4⁺ cells, 15–20% B cells, and 10–15% CD8⁺ T cells, while in the lacrimal glands, the populations are skewed slightly in favor of the B cells (25–30%), the Th cells constituting approximately 25–30%⁵⁶. Of note, unlike the human pathology, the NOD parotid glands remain free of lymphocytic infiltrates. The infiltrating T cells express restricted Vβ usage in both tissues, with an increased use of Vβ₆ and Vβ₈⁴⁸. While focal infiltrates will develop at different times depending on the animal model studied (and certainly in Sjögren's syndrome patients), comparisons of the NOD and MRL/lpr strains indicate that the autoimmunity in the NOD model, but not the MRL/lpr model, is preceded by an influx of CD11c⁺ dendritic cells⁵⁶.

The analysis of aberrant apoptotic activity in the diseased exocrine glands has led to several interesting observations regarding the focal immune infiltrates. While various studies have shown elevated levels of apoptosis in the salivary exocrine tissues in association with disease onset in both Sjögren's syndrome patients and in the various mouse models^{9, 30, 46}, there is also data suggestive that the immune infiltrates are excessively resistant to the induction of programmed cell death. First of all, these populations appear to have upregulated bcl-2^{21, 28}, which may contribute to their protection against programmed cell death induced by upregulated expression of FasL in the salivary tissues³⁰ and, subsequently, an aberrant persistence in the exocrine glands. This is in marked contrast to the documented upregulation of Bax and caspase-3 in the salivary acinar cells³⁷.

The exact role of the infiltrating populations remains obscure; however, the importance of the individual subpopulations is well documented. Adoptive transfer studies have demonstrated the ability of splenic T cells from diseased NOD mice to transfer sialadenitis

into neonates²¹, suggesting that the T cell compartment of the immune response is able to drive the underlying histopathology observed in Sjögren's syndrome. This is further supported by our recent findings in the context of the NOD.IFN- γ mouse model that histopathology and secretory dysfunction do not occur in the absence of IFN- γ , a classic inflammatory cytokine (unpublished data). Recent evidence also suggests that, just as in the case of autoimmune diabetes in the NOD background^{14, 50}, B lymphocytes are essential for initiation and effector phases of autoimmune exocrinopathy. NOD.Ig μ^{null} mice, deficient in B lymphocytes, develop only sparse infiltrates and maintain full secretory capacity³⁸. This also emphasizes the fact that glandular immune infiltration by T cells alone is not sufficient to drive the glandular hypofunction, although this does not entirely rule out the possibility that T cells may contribute to glandular suppression in a B cell dependent fashion, requiring the B cell as an antigen-presenting cell and thereby leading to the unique activation of an effector T cell subpopulation. Nonetheless, the passive transfer of patient- or NOD-derived IgG induces exocrine cell suppression in the B cell-deficient NOD mouse, demonstrating the significant and dominant role of autoantibodies in this capacity³⁸.

Expanding Our Understanding

Our appreciation of the immunological component in Sjögren's syndrome has greatly improved based on the studies of several mouse models of autoimmune exocrinopathy. Unfortunately, in our attempts to dissect the significant events in the immunological attack on the exocrine tissues, there is a tendency to focus too narrowly on a specific gland, typically either the lacrimal or salivary glands, rather than assessing the global effects in all of the affected tissues unilaterally. The oral and ocular manifestations of Sjögren's syndrome share many similar underlying factors, despite the existence of several fundamental differences such as slight variances in the expression of cytokines. The fact that the development, of Sjögren's syndrome in patients may effect both the lacrimal and salivary glands, or target one of these exocrine glands independently, implies that there is still a need to continue focusing, in part, on the individual glandular pathologies. However, such intriguing findings as the importance of IL-4 emphasize the point that simply looking at the pathogenesis within the exocrine glands fails to take into consideration many essential factors occurring on a more global systemic immunological level. In fact, of the

autoimmune pathologies reliant on an effector autoantibody, pemphigus vulgaris is one of the few that is associated with the detection of IL-4 at the site of immune-mediated damage, while autoimmune diseases such as Grave's disease and Hashimoto's thyroiditis, dependent at least in part on the presence of effector autoantibodies similar to Sjögren's syndrome, are historically associated with a pro-inflammatory cytokines such as IFN- γ in the glands^{7, 12, 57}. Many of the critical events involved in orchestrating the immunological attack occur in the draining lymphatics, not in the tissue lesion. Certainly, all evidence points to this scenario being the case in terms of Sjögren's syndrome. For instance, the antibodies shown to mediate exocrine suppression are produced by plasma B cells that likely reside in the luminal regions of the draining lymph nodes or in the bone marrow despite the evidence supporting locally produced IgM and IgG by infiltrating B cells. This does not rule out the importance of the exocrine lymphocytic infiltrates in the overall disease state, but it also implies that important effector populations reside in extraglandular locations. Therefore, conclusions drawn from the analysis of the disease state strictly within the exocrine tissues, such as cytokine profiles, do not provide enough information to understand truly the mechanisms of the disease.

Over the last few years, new strategies have been applied to Sjögren's syndrome research with the aim of identifying cytokine production associated with the invading leukocyte populations. Rather than simply measuring mRNA message levels (especially with the recent appreciation for inconsistencies involving mRNA message versus protein expression), investigators have been increasingly reliant on isolating clonal T cell populations to identify Th cytokine expression profiles. The conclusions generated from this approach have provided significantly different information and have brought much of the mRNA-based cytokine data into question with respect to several autoimmune diseases including Sjögren's syndrome and Grave's disease^{36, 61}. However, it is critical to point out that any conclusions drawn from cell culture experiments must be treated with a certain degree of skepticism, due to the inherent nature of lymphocytes to adjust quickly to their environment resulting in altered clonal selection within the cell population or even changed commitment to a Th pathway.

Looking more closely at Sjögren's syndrome, cytokine mRNA analyses within the target tissues repeatedly suggest an absence of IL-4 despite the development of a critical humoral component in the disease pathogenesis, not to mention the development of one of the hallmark features of Sjögren's, B cell hyperplasia

and hypergammaglobulinemia. In contrast, the isolation and study of T cell clones has brought to light the existence of Th0 and Th2 subpopulations secreting IL-4 in the tissue lesions³⁶. This is in agreement with the observations recorded in the characterization of the IL-4 knockout model of Sjögren's syndrome. If T cell clonal isolation and expansion experiments are to be believed, then MATSUMOTO, et al.³⁶ may have identified a potential source for the IL-4 that appears to be essential in autoimmune exocrinopathy in the NOD mouse. Nonetheless, it is important to consider that *in vitro* clonal expansion of isolated T cells may introduce alterations into the commitment patterns of these cells to a specific Th profile¹ and *in vitro* culture systems can easily be manipulated to force this change³³. Furthermore, the presence of Th0 cells in the glandular lesions at the time of cell isolation, which may simply be at an earlier non-committed stage of development, may serve as a possible source of experimental artifact with respect to the presence of T lymphocytes in the lesions exhibiting a Th2 profile *ex vivo*. In retrospect, the mRNA expression data derived from tissue samples may call *in vitro* clonal expression data into question.

Finally, this approach still perpetuates one of the most difficult challenges in the understanding of Sjögren's syndrome, which is to enhance our comprehension of the global multi-organ disease state as it exists in the patient. Infiltrating lymphocytic populations isolated from lacrimal versus salivary gland lesions will no doubt be different, simply based on the influence the target tissues exert on the immune response as evidenced, in part, by the specific infiltration of the lacrimal glands but not the salivary glands in the NOD.IFN- γ mouse (unpublished observation). But in the chronological order of events leading to the activation of the autoimmune lymphocyte, the exocrine gland is only one of the critical microenvironments that provide essential maturation signals. In conclusion, while these approaches tend to provide significant insights into the mechanisms, we cannot possibly expect to understand the true pathogenesis of Sjögren's syndrome until we begin to consider not just what cells or cytokines are present, but also when and where cytokines influence cell development, and the sequence of events, microenvironments, and cellular interactions that critically influence the maturation of the adaptive immune response that mounts a devastating attack against the exocrine glands.

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