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Immunopathogenesis of Keratoconjunctivitis Sicca in the Dog

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Keratoconjunctivitis sicca (KCS), more commonly known as dry eye, is an inflammatory condition of the ocular surface caused by pathologic reduction in the aqueous component of the tear film. It is seen commonly in the dog and defined as a Schirmer tear test (STT) reading of less than 10 mm/min with concomitant ocular surface pathologic findings [1]. KCS can be divided into two types: one in which tear production is deficient and other cases in which tear evaporation accounts for the ocular surface tear deficiency. In the dog, the latter is seen in brachycephalic dogs in which lagophthalmos, that is to say failure of complete eyelid closure, leads to a central area of tear film deficiency or in dogs in which a deficiency in tear film lipid leads to increased tear loss through evaporation. In this review, the author concentrates on the former condition in which pathologically reduced aqueous tear production leads to the ocular pathologic findings of corneal vascularization, pigmentation, and, in several cases, frank ulceration (Figs. 1–3).

The first attempt to document the prevalence of dry eye in canine keratoconjunctivitis sicca (cKCS) was undertaken in a university clinic population more than 30 years ago [2] and suggested a figure of 0.4% of animals affected, whereas a more recent report documented a prevalence as high as 35% in 460 dogs [3]. The author and his colleagues [4] have performed STTs on 1000 randomly selected animals from the general canine population and determined that 4% had STT values less than 10 mm/min. Clearly, cKCS is a common and probably underrecognized condition in the general canine population. Its treatment has been revolutionized over the past 15 years by the introduction of topical cyclosporine as an effective lacrimogenic agent [5], yet the etiopathogenesis of the condition is still poorly understood. The same holds true for dry eye in human beings, which is a widely recognized problem in postmenopausal

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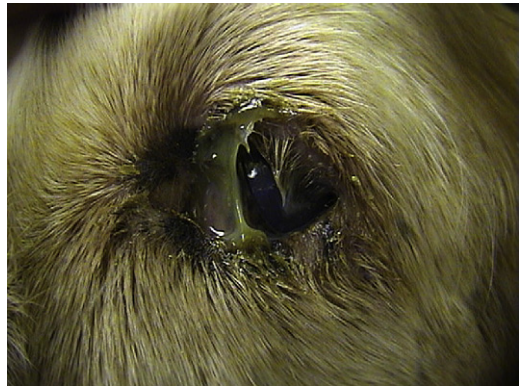

Fig. 1. This 6-year-old West Highland white terrier neutered bitch shows the characteristic appearance of canine keratoconjunctivitis sicca (cKCS), with sterile mucoid discharge as the predominant feature.

women. In some of these cases, it is associated with other exocrinopathies, such as xerostomia from salivary gland involvement, and more generalized connective tissue diseases, such as rheumatoid arthritis, in which case it is known as Sjögren's syndrome (SS); however, in many cases, it exists on its own as an uncomfortable ocular surface condition for which optimal long-term treatment is taxing. Rodent models abound, even though measuring tear production in rats and mice is difficult. The purebred mouse strains mirroring SS have allowed considerable dissection of the immunopathogenesis of the disease, yet how

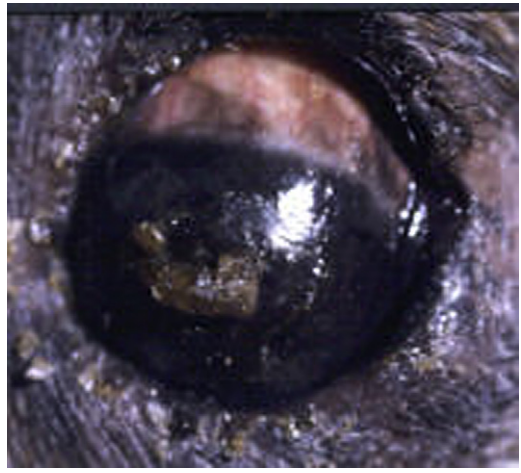

Fig. 2. In this 12-year-old pug with tear deficiency and evaporative dry eye, the desiccated and hyperpigmented ocular surface shows the classic signs of chronic canine keratoconjunctivitis sicca (cKCS).

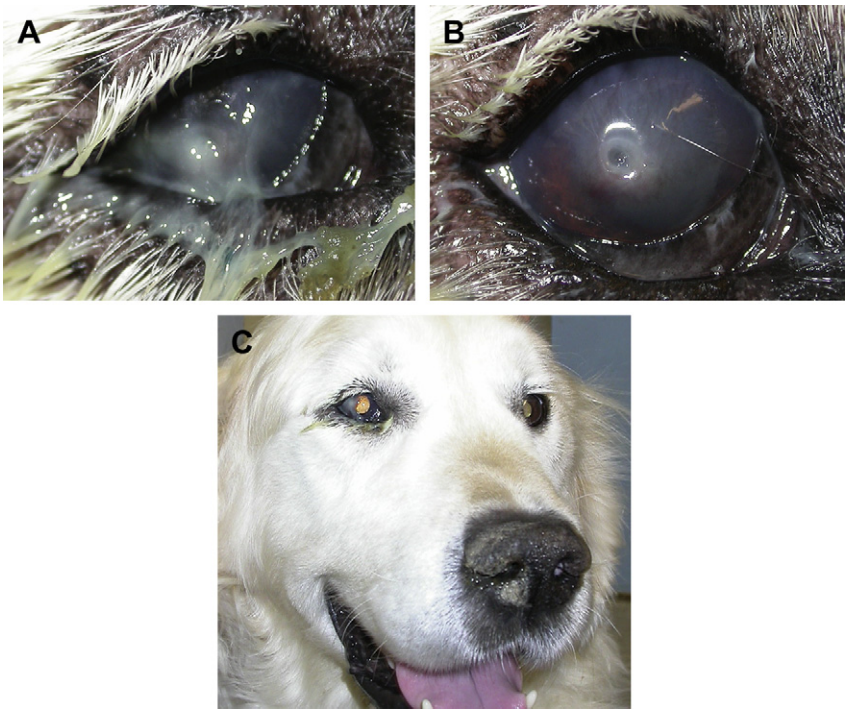

Fig. 3. In this 9-year-old golden retriever, canine keratoconjunctivitis sicca (cKCS), again characterized by profound mucoid discharge (*A*) has led to a central deepening corneal ulcer (*B*), which is only seen once the discharge has been cleared from the eye. (*C*) Tear deficiency is neurologic in origin, as seen by the dry external nares. The dog has otitis media, which accounts for the neurologic cKCS.

much this similarity holds for human beings and dogs is somewhat unclear. The nonobese diabetic (NOD) mouse and the MRL and MRL/lpr congenic strains are important rodent models for human SS, and also potentially for cKCS, whereas other strains, such as the transforming growth factor (TGF)-β1 knockout mouse and New Zealand Black hybrid mouse, may also provide valuable models for immune-mediated tear deficiency. In this review, the author provides some novel findings regarding the immunopathogenesis of cKCS, discusses immunologic abnormalities in human KCS and SS, and assesses the contributions made by research on the rodent models outlined previously, explaining how understanding of these noncanine examples can inform our treatment of the disease in the dog.

ANATOMY AND PHYSIOLOGY OF TEAR PRODUCTION

For many years, the tear film has been characterized as having three layers: a mucus layer close to the corneal epithelial surface, an aqueous layer, and,

finally, a most superficial layer of meibomian lipid that acts to reduce evaporation [6]. More recently, studies have shown that this cut-and-dried distinction between layers is not strictly correct–the corneal epithelium has a surface glycocalyx composed of membrane-spanning mucins (including MUC1, 3, 4, 12, 13, and 16), with these have signaling capabilities through their cytoplasmic tail and extracellular epithelial growth factor-like domains [7]. The aqueous layer, far from being solely aqueous in nature, is filled with cleaved membrane-bound mucins, small soluble mucins (MUC 7 and 9), and much larger gel-forming mucins (including MUC2, 5, 6, and 19) [8]. Mucins are formed in conjunctival goblet cells, whereas tear film lipid arises from the lid meibomian glands. The aqueous component of the tear film is produced in the dog from the lacrimal gland dorsolateral to the globe and is closely associated with it; it is also produced by the gland of the nictitating membrane. Recently, it has become clear that to understand the physiology of tear production and the pathophysiology of dry eye, the entire unit of the lacrimal glands, ocular surface, and innervation connecting them needs to be considered fully to describe the pathologic events occurring in KCS. Although this review's discussion of immunopathogenesis primarily concerns the glandular tissue producing the aqueous component of the tear film, we must not forget that this is merely a part of this "lacrimal functional unit" [9].

Lacrimal gland secretion is under neural and hormonal control; the lacrimal gland has parasympathetic and sympathetic innervation with a reflex arc identified from sensory nerves in the cornea activating efferent parasympathetic and sympathetic nerves originating in the parasympathetic motor nucleus of the facial nerve but traveling with the trigeminal nerve to the lacrimal gland. The neurotransmitters acetylcholine, vasoactive intestinal peptide, substance P, noradrenaline, and calcitonin gene-related peptide are all important in lacrimal secretion, but acetylcholine and noradrenaline are the most potent stimuli to water and electrolyte secretion in tears [6]. Hormonal control by means of the hypothalamopituitary-gonadal axis has a profound effect on tear secretion, with adrenocorticotrophic hormone, α-melanocyte–stimulating hormone, prolactin, androgens, estrogens, and progestagens all having an influence on lacrimal gland function [6]. In particular, androgens are potent stimulators of tear production with important gender- and age-related effects on tear production.

OPHTHALMIC EXAMINATION OF THE TEAR FILM

Standard direct ophthalmoscopy and indirect ophthalmoscopy are important in the evaluation of the ocular surface and tear film. In particular, evaluation of the reflection of a focal light source shone onto the ocular surface demonstrates the integrity of the epithelial surface and the tear film (see Fig. 1). The normal measure for tear production in the dog is the STT, alone (STT I) or after topical anesthetic (STT II), with these being well recognized and reported in the veterinary literature [1,10,11]. Commonly used to measure tear production, the STT, in reality, assesses a combination of the rate of tear production and the volume of the tear lake [12]. The phenol red thread test provides less of

a stimulus to lacrimation and is more appropriate for small mammals than is the STT but has yet to find a place in routine assessment of the canine tear film [13]. In human ophthalmology, the STT is used in combination with several other methods of assessing the tear film, which should probably play a wider part in investigation of canine lacrimation. Tear film breakup time is becoming more commonly used to assess the combination of reduced tear production and increased evaporation in ocular surface tear film deficiency [14]. Determination of the tear volume by meniscometry has been championed by some ophthalmic researchers [15] but has yet to be reported in the veterinary sphere. Rose bengal staining assesses pathologic changes to the epithelial surface [16] but has yet to be used sufficiently in canine ophthalmology to allow correlation of the test with other measures of canine tear deficiency. The meibomian gland plays an important part in providing the lipid that reduces tear film evaporation, and assessment of meibomian gland function has recently been reported in the normal dog [17], but changes in animals with cKCS have yet to be defined.

Overall, the STT is still an important criterion by which to judge ocular surface health, with readings less than 10 mm/min indicative of dry eye. All too often, the problem is not that other tests for tear production should be used in ocular examination but that the STT is not used sufficiently in general veterinary practice. The author and his colleagues [4] have shown in their study of 1000 dogs that the average STT reading is 18.6 mm/min and that for the breeds classically associated with cKCS, the tear production even in normal dogs is significantly lower. Increasing age is associated with decreasing production of tears [18], and in a study of dogs with endocrinopathies, the author and his colleagues [19] have demonstrated that animals with diabetes mellitus, hyperadrenocorticism, and hypothyroidism all have reduced tear production. Thus, older dogs of such breeds as those in Table 1; dogs with the previous endocrinopathies; and any dog with corneal ulceration, corneal inflammation, or a red eye should be subject to careful ocular surface evaluation, including the STT.

IMMUNOLOGIC ASPECTS OF CANINE KERATOCONJUNCTIVITIS SICCA

Early research on the etiopathogenesis of cKCS centers around the work of Dr. Rene Kaswan in the 1980s. The first report of Kaswan and colleagues [20] documented histologic evaluation of 40 nictitating membrane glands (NMGs) and 9 main lacrimal gland (MLGs) from 28 dogs affected by cKCS with varying diagnoses of concurrent systemic disease from distemper, diabetes mellitus, systemic lupus erythematosus (SLE), and hypothyroidism. This population differs substantially from the case load seen by the current author, in which most dogs have KCS as their only immune-mediated disorder. In the study by Kaswan and colleagues [20], 20% of the NMGs and 22% of the MLGs had insufficient glandular tissue on biopsy for evaluation, whereas the remaining histologic findings in 10% were considered within normal limits.

Table 1
Canine breeds predisposed to keratoconjunctivitis sicca

Breed	Relative risk
Cavalier King Charles spaniel	11.5
English bulldog	10.8
Lhasa apso	9.8
Shih tzu	6.2
West Highland white terrier	5.5
Pug	5.2
Bloodhound	4.5
American cocker spaniel	4.1
Pekingese	4.0
Boston terrier	2.0
Miniature schnauzer	1.8
Samoyed	1.7
Other breeds	1.0

Data from Kaswan RL, Salisbury MA. A new perspective on canine keratoconjunctivitis sicca. Vet Clin North Am Small Anim Pract 1990;20:595.

Sixteen percent of glands were classified as having stage I inflammatory disease with minimal numbers of periductal and periacinal lymphocytes and some fibrous connective tissue replacing tubuloacinar elements. Thirty-three percent of glands exhibited stage II inflammatory change (Fig. 4) with lymphatic nodules, squamous metaplasia of ductal epithelial lining, and, in dogs with distemper only, the presence of numerous neutrophils. Thirteen percent of glands were classified as having stage III inflammatory change replacement of glandular elements with fibrous connective tissue and moderate numbers of

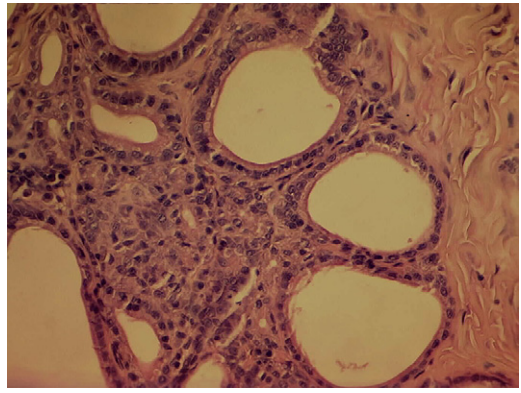

Fig. 4. Mild inflammatory cell infiltrate and fibrosis in the lacrimal gland of a West Highland white terrier with cKCS.

mononuclear inflammatory cells. Duct dilation and epithelial squamous metaplasia were seen in several dogs.

More recently, Izci and colleagues [21] showed a significant decrease in CD8+ lymphocytes and reversal of the CD4+/CD8+ ratio in NMGs of dogs with KCS after 30 days of treatment with topical 2% cyclosporine giving concurrent regression of clinical signs. The study, however, failed to include information on the initial lymphocyte populations in the affected NMGs. The author and his colleagues [22] have recently performed immunohistochemistry on NMG biopsies from nine dogs with normal STT values, six with idiopathic cKCS, and two with cKCS associated neurologic etiopathologic findings characterized by dry nostrils and dry eyes. The number of CD3+ T lymphocytes as a proportion of cells in the lacrimal tissue in normal dogs was 0.058 compared with 0.143 in dogs that had idiopathic KCS and 0.079 in samples from dogs that had neurogenic KCS (Fig. 5). The proportion of CD79a-expressing B cells in the NMG of dogs with normal tear production was 0.087, whereas in dogs that had idiopathic KCS, it was 0.181, and in neurologic cases of KCS, the proportion was 0.202. Numbers of T and B cells were significantly increased in idiopathic cKCS ($P = .002$ and $P = .044$, respectively) but not in neurologic cKCS ($P = .07$ and $P = .18$, respectively). These results show that the increase in T-lymphocyte numbers is likely to be the cause of the disease and not the result of ocular surface drying, in which case, numbers would be significantly increased in idiopathic (ie, presumed to be immune-mediated) and neurologic cases. Unfortunately, we do not currently have access to antibodies against CD4 and CD8 epitopes in canine fixed embedded tissue, and thus cannot evaluate T-lymphocyte subpopulations at present. The inflammatory infiltrate in these cases is periacinal, presumably directed at antigens on acinal cells of the lacrimal glandular tissue. This is not, however, the only mechanism of reduction in tear production.

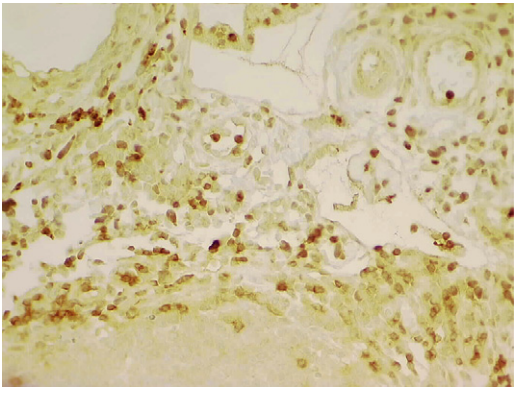

Fig. 5. Immunohistochemistry shows CD3-expressing T cells in the periacinal tissue of the lacrimal gland in a cocker spaniel with cKCS.

cKCS in leishmaniosis is the result not of periacinal inflammation but of periductal infiltration [23], with constriction of the outflow of tears and subsequent dilation of lacrimal ductules (Fig. 6). Quite what the inciting antigen resulting in this periductal lymphocytic infiltrate is remains unclear, but it is unlikely to be the same as that enticing cellular infiltration in idiopathic immune-mediated cKCS.

Kaswan and colleagues [24] continued work on the immunology of cKCS with reports documenting systemic immunologic changes in the disease—autoantibodies against undefined lacrimal antigens in one article and rheumatoid factor in another [25]. Autoantibodies are important in human SS as detailed elsewhere in this article and in rodent models of immune-mediated KCS. As such, the nature of autoantibodies in cKCS should be more carefully elucidated. In the report of Kaswan and colleagues [24], 40% of the cases had concurrent immunologic disease, such as SLE, pemphigus foliaceous, or rheumatoid arthritis, or diseases with a possible immunologic component in their etiopathology, such as hypothyroidism or diabetes mellitus, generalized demodectic mange, ulcerative colitis, or glomerulonephritis; thus, again, these cases may not reflect the population of dogs we see with cKCS, in which, generally, tear abnormality is the only complaint. Five of 9 dogs tested had autoantibodies against lacrimal antigens on direct immunofluorescence, whereas 9 of 31 dogs demonstrated autoantibodies to ductal tissue in the NMG. Sixty-seven percent of dogs had hypergammaglobulinemia, and 16% had elevated serum IgA. Forty-two percent of dogs tested had circulating antinuclear antibodies. The relevance of these findings in understanding the pathogenesis of cKCS is unclear, because it is impossible to say whether such immunologic changes are a cause or effect of lacrimal inflammation. Clearly, more work is needed to repeat and extend this work.

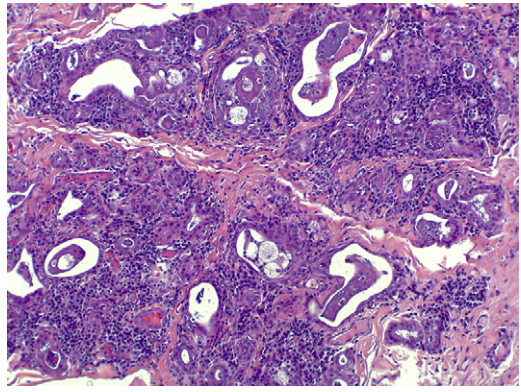

Fig. 6. Periductal inflammatory cell infiltrate in a dog with cKCS associated with leishmaniosis. (*Courtesy of* Teresa Peña, DVM, PhD, Barcelona, Spain.)

It has to be added, however, that apart from the ductal occlusion in leishmaniosis, the data presented here do not explain why tear production should be lower. In many cases, neurologic cKCS is also characterized by a dry nose (Fig. 3C) because the innervation to the medial nasal gland giving wetting of the external nares is also affected. It is relatively straightforward to explain how efferent denervation impairs lacrimal secretion, but the exact mechanism by which inflammation reduces tearing is less easily understood. For research attempting to explain this, we need to examine the work on rodent models presented elsewhere in this article, with discussion of the debate that still rages regarding the mechanism of glandular hypofunction in SS. Before that, however, the treatment of cKCS requires discussion.

TREATMENT OF CANINE KERATOCONJUNCTIVITIS SICCA

A key feature suggesting that immunologic changes are central to the etiopathogenesis of cKCS is the ameliorative effect of cyclosporine, a drug first used to treat transplant rejection in human patients [26] and now employed as a systemic immunomodulator in canine patients that have immune-mediated conditions as diverse as atopy [27], anal furunculosis [28], and myasthenia gravis [29]. Topical use of this drug started at 2% [5,30] and was reduced in later studies to 1% [31], and, finally, in the commercially marketed medication Optimmune (Union, New Jersey), to 0.2% [32]. Cyclosporine is a specific immunomodulator that prevents lymphokine production through its action as a calcineurin inhibitor [33], whereas other more recently developed agents within this family of drugs, such as tacrolimus [34] and pimecrolimus [35], have been suggested to have a more potent effect, although our findings are that in cases resistant to the lacrimogenic effects of 2% cyclosporine, topical tacrolimus, at least, shows little added clinical benefit [36], whereas other investigators have somewhat contrary findings [34], with tacrolimus yielding a better lacrimomimetic effect than cyclosporine. It has to be said that these two results are not mutually exclusive–one might say that although the lacrimomimetic effect of tacrolimus is clearer than that of cyclosporine when some remaining glandular tissue exists, when there is no exocrine glandular tissue left and lacrimal gland pathologic change has reached the fibrotic stage III of the study by Kaswan and colleagues [20], no immunomodulatory lacrimogen acts to increase tear production. It is at that stage that parotid duct transposition is the only effective therapeutic way forward [37]. The original hypothesis that the lacrimomimetic effect of cyclosporine is through a local immunosuppressant is called into question by its lacrimogenic effects in dogs without cKCS, in which tear production is increased [33], and by experimental studies on neuromodulatory effects [38], which are discussed further elsewhere in this article. Other work on dogs rendered lacrimally deficient by removal of the NMG and the MLG demonstrated that the drug has effects on mucus production and the ocular surface separate from those on aqueous secretion [39].

Another immunomodulatory treatment is that using α-interferon (IFN) orally [40]. Just more than half of the 20 treated animals showed a favorable

response to an escalating dose regimen of cytokine administration from 20 to 80 IU/d. Similar beneficial effects were noted with regard to lacrimal and salivary secretory function in human patients who have SS [41]. Given that the cytokine is now recognized to act as a balancing agent in the immune system [42], such treatment has a firm rationale, although little further development of α-IFN seems to have been undertaken after the preliminary report by Gilger and colleagues [40].

Finally, the use of parasympathomimetics should not be forgotten. Topical use of pilocarpine at concentrations between 0.25% and 2% caused blepharospasm, conjunctival hyperemia, and miosis of the treated eye without significant increase in tear production [43], whereas systemic administration by use of drops applied on the tongue stimulates tear production in normal dogs and in a proportion of KCS-affected animals [44].

MECHANISMS OF TEAR HYPOSECRETION: AN INTRODUCTION

Quite apart from the diagnosis and treatment of cKCS, there remains the rather more academic question concerning the mechanism by which the inflammatory changes in KCS more generally lead to a profound reduction in tear production. One suggestion is that lymphocyte-associated cytotoxicity of lacrimal tissue is central to the pathologic effects on lacrimation. A second is that apoptosis of glandular epithelial cells is critical in tear hyposecretion. A third is that cytokine release from inflammatory cells alters tear production. Finally, inflammatory cells or their associated cytokines or autoantibodies may influence neurotransmitter function in the lacrimal gland, inhibiting neurologic stimulation of tear secretion. These possible mechanisms are not, of course, self-excluding, and more than one may be in action in one or several of the tear deficiency syndromes seen in dogs, in human beings, and in experimental animal models. It is with these possible routes to tear deficiency in mind that the reader is invited to consider the immunopathologic research in human beings and rodents detailed here.

IMMUNOPATHOLOGY OF KERATOCONJUNCTIVITIS SICCA IN HUMAN PATIENTS

Similar pathologic changes to those described previously for cKCS are seen in the lacrimal and salivary glands of human patients who have SS: CD4+ T cells predominate in the lesions, with increased major histocompatibility complex (MHC) class II expression on glandular epithelial cells [45,46]. These T cells express a limited number of T-cell receptor phenotypes shared between lacrimal and salivary glands, suggesting that they are clonally expanding in response to a shared antigen between these two glands [47]. Several cell lines cloned from infiltrated salivary gland tissue showed autoreactivity to the Ro/SSA 52-kDa antigen against which autoantibodies are present in affected patients [48]. Ro/SSA is a ribonuclear polypeptide complex, whereas La/SSB is

a 48-kDa protein against which autoantibodies are also directed in SS, SLE, and several other connective tissue autoimmune diseases. These proteins are normally nonimmunogenic but can associate with stress proteins, such as calregulin, which, although normally regulating calcium homeostasis in the endoplasmic reticulum (ER), when overexpressed, increases calcium levels in the ER and aberrantly protects the cell from apoptosis [49]. Interaction between these ribonuclear polypeptides and calregulin may result in failed clearance of apoptotic debris by macrophages and provoke a cascade of autoimmune reactions that lead to connective tissues diseases, such as SS and SLE.

Ironically, although the CD4+ hyper-T cell is reported as central to the pathogenesis of SS, there is also evidence that the disease is primarily a B-cell–dominated condition [50]. B cells can differentiate to have the characteristics of T helper (Th) cells, secrete Th1 or Th2 cytokines, and thus be termed B-effector (Be) lymphocytes. It may be that these cells are instrumental in SS pathogenesis. Rituximab, a humanized anti-CD20 (and thus anti-B cell) monoclonal antibody, has a profound ameliorative effect on several patients who have SS, showing how important B cells are in disease pathogenesis. Indeed, in some patients, there is a fine line to be drawn between reactive B-lymphocyte expansion in SS and mucosal-associated lymphoid tissue (MALT) lymphoma [51]. Generally, autoimmune diseases are characterized by lesions in which Th1-lymphocyte populations predominate, with cytokines, such as interleukin (IL)-1, IL-2, and γ-IFN. Some researchers find these cytokines in SS lesions [52], whereas others, working with lesions at a different stage of disease progression, find Th2-cell populations [53]. Indeed, the most recent work published at the time of writing this review shows different lymphocyte populations at different time points in the development of disease [54]. The Th2 cytokines IL-4 and IL-5 are seen in early lesions, although as the inflammation progresses and worsens in severity, the balance swings in favor of Th1 cytokines and a more aggressive cytotoxic immune response. Quite how this influences lacrimal function, and what it means for treatment options, remains unclear at present.

LABORATORY MODELS OF KERATOCONJUNCTIVITIS SICCA

Several rodent models of SS and its associated KCS are available, and each adds to the overall understanding regarding pathogenic mechanisms in human SS-associated KCS and also, potentially, in cKCS. A good review is that provided by Barabino and Dana [55]. In this article, the author concentrates on the immunologic aspects of the MRL+, MRL/lpr, and NOD mice.

For many years, the MRL+ mouse strain has been recognized as a model for human SS. As in human patients who have SS, female mice are more susceptible to infiltration of lacrimal tissue by T lymphocytes [56]. A more aggressive lacrimal gland inflammation develops in the MRL/Mp-lpr/lpr strain in which the lpr gene, encoding the proapoptotic fas protein, is defective [57]. Lymphocytes in this mouse strain fail to apoptose, that is, to commit programmed cell death; thus, massive accretions of activated lymphocytes develop

in several tissues, particularly in the lacrimal glands. Apoptotic cell death of lacrimal gland epithelium may be important in the initiation of disease in other mouse models, as discussed further elsewhere in this article. Note the potential similarity to alterations in apoptosis in the human patients as discussed previously.

T-cell populations in the MRL/lpr mice are CD4+ in character [58], and antibodies against CD4 ameliorate disease [59]. Cytokines in these lacrimal gland lesions are predominantly Th2 in character with IL-4 RNA transcripts being present in between 100 and 1000 times great number by reverse transcriptase polymerase chain reaction (RT-PCR) than γ-IFN transcripts. Glandular epithelial cells, which can act as nonprofessional antigen-presenting cells [60], express the costimulatory molecule B7-2 (CD86), which is associated with generation of a Th2 response, rather than B7-1 (CD80), which produces a Th1 inflammatory phenotype [61]. Were it to be thought that this indicates a benign antibody-mediated immune response rather than an aggressive cytotoxic reaction, it must be remembered that the proinflammatory mediators nitric oxide and tumor necrosis factor (TNF)-α have also been detected in these lesions [62,63]; thus, not everything on the lacrimal front is quiet in the disease.

Lacrimal inflammatory disease in the NOD mouse offers another autoimmune murine model of SS with a Th1 CD4 lymphocyte inflammatory cell population [64]. Several proinflammatory cytokines, including IL-1β, IL-6, IL-7, IL-10, γ-IFN, and TNFα, were detected, together with inducible nitric oxide synthase (iNOS), but IL-4 synthesis was absent in lacrimal and salivary glands. Cytokines detected in lacrimal tissue appeared earlier and more intensely in the submandibular glands [65,66]. Interestingly, other research groups find that defective neurotransmitter signaling in the lacrimal and salivary glands of these NOD mice precede the inflammatory infiltrate in these mice [67]. Other changes within the gland may be nonimmunologic in origin. In NOD–severe combined immunodeficiency (SCID) transgenic mice, in which the NOD genotype occurs in the absence of T or B cells, there are changes in the lacrimal gland with age [68]. NOD-IgM null mice, which lack B cells but still have T cells, do not lose secretory function even though they have a T-cell infiltrate in their lacrimal glands [69]. Autoantibodies to M3 acetylcholine receptors have been shown to play a pivotal role in the reduction of glandular secretion in NOD mice, as is discussed further elsewhere in this article [70,71], and complement also plays an important part in disease pathogenesis [72].

Two questions remain after this large volume of work on the immunologic phenotype of these dry eye mouse models. One must continue to enquire as to the nature of the initial autoantigen that sets the inflammatory process off. Second, we have much still to learn regarding the link between the inflammatory process occurring in all these individuals, be they rodents, dogs, or people, and the reduction in secretory output that is the key feature leading to clinical disease of the ocular surface. Perhaps the M3 muscarinic receptor lies at the heart of the answers to both of these questions.

MECHANISMS OF TEAR HYPOSECRETION: REVIEWING THE EVIDENCE

The classic model of glandular dysfunction or hypofunction regards the loss of gland secretion as being caused by immune destruction of gland tissue and subsequent apoptosis of glandular epithelium [73]. Yet, although apoptosis has been reported in dogs, people, and rodents with KCS [74], it is not an invariable finding. Perhaps more relevantly, in many cases of human SS and in a sizeable proportion of dogs with cKCS, a significant amount of glandular tissue appears apparently normal. Laboratory studies show this tissue to be functional in vitro [75], and cases unresponsive to immunomodulators, such as cyclosporine, may revert to normal function with application of systemic parasympathomimetic lacrimologues [76]. Defective neurotransmitter-mediated signaling has been described in the NOD mouse [67], and other researchers have suggested that such defects point to a nonimmunologic factor in the etiopathogenesis of dry eye [68]. Elevations in levels of matrix metalloproteinases in the disease again point to different disease processes occurring concurrent with the inflammatory infiltrates, but determining whether these are a causative agent or an effect of inflammatory change is problematic [77].

What predisposing abnormalities actually initiate the disease? Clearly, the genetic background is important, not to say central. Although we do not yet know the dog leukocyte antigen haplotypes of predisposed breeds, as listed in Table 1, it is likely that these breed predispositions mirror the population genetics of human SS patient groups in which the human leukocyte antigen (HLA) haplotypes HLA-Dw3 and HLA-B8 are associated with SS disease [78]. Hormonal changes reflected in the gender assignment of most patients who have SS and in MRL+ and MRL/lps mouse models (but interestingly not in the NOD mouse) [79] have a critical part to play in the generation of dry eye in most rodent models, in human patients who have SS and non-Sjögren's dry eye, and in dogs [18]. Androgen deficiency is a key feature of human SS [80] and mouse models of the disease, in which testosterone treatment reduces the disease severity, increasing tear secretion and IgA content [81]. Androgen receptors have been reported in the acini of the glandular epithelium and not within the inflammatory cell infiltrate in lacrimal lesions in mouse models, such as the MRL/lpr mouse [82], suggesting that the response to androgen therapy is primarily caused by glandular physiologic changes and not initially by an amelioration of the inflammatory pathologic changes noted. Having said that, the same research group has previously reported that androgen treatment does markedly reduce the inflammatory cell population in the lacrimal gland [83]. Proapoptotic genes, such as pcl-2, are expressed in the lacrimal gland of these mice in a strikingly gender-specific manner [84], with this influenced by testosterone administration. In addition, normal organogenesis of the salivary gland has been reported in NOD mice, which progresses to inflammatory disease of this structure, suggesting that there may be initial nonimmunologic factors involved in disease pathogenesis [85], but similar results have not been reported for the lacrimal gland.

A hypothesis that draws these divergent strands of disease pathogenesis together might be one that sees the effects of antimuscarinic receptor autoantibodies on neurotransmitter release, giving the secretory failure and also provoking a further inflammatory response that eventually leads to glandular destruction. Defects in apoptosis may rest at the heart of the original autoantigenic challenge to the immune system, with apoptosis of secretory epithelial cells exposing previously cryptic autoantigens, such as α-fodrin, a calmodulin-binding protein, to the immune system [86]. The lymphocytes apparently at the heart of SS congregate around acini with apoptotic cells [87], and antigens unmasked in apoptotic fragments seem to be important in lymphocyte-associated cell death [88]. The occurrence, or lack of it, of autoimmune responses in mouse models seems to be linked to display and cleavage of autoantigens in apoptotic cells in work from some researchers [89], whereas other investigators propose a model for lacrimal hypofunction that does not involve apoptosis [90] but in which local neurologic disturbance, caused primarily by proinflammatory cytokines, explains most of the clinical and immunohistologic signs seen in the disease.

The truth of the matter probably lies in a combination of these mechanisms, with various factors (genetic, environmental, apoptotic, and, possibly, infectious) leading to the exposure of self-antigens, production of danger signals as first suggested by Matzinger [91], and development of an autoantibody-mediated inhibition of neurotransmitter function giving tear deficiency [92].

SUMMARY

It may be possible to confirm such a train of pathologic events as outlined here in rodent models and even in human patients who have SS; however, as we have seen here, cKCS languishes a long way behind in research terms even if, as veterinarians, we lead the field, and have done so for nearly 2 decades, in effective lacrimogenic treatment for cKCS in the form of topical cyclosporine. We have not yet even fully characterized the inflammatory cell population in the lacrimal glands of dogs affected by cKCS or evaluated their genetic basis. It is hoped that this review spurs further research into the etiopathogenic factors in cKCS.

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