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Exfoliative cutaneous lupus erythematosus in German shorthaired pointer dogs: disease development, progression and evaluation of three immunomodulatory drugs (cyclosporin, hydroxychloroquine, and adalimumab) in a controlled environment

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Abstract

Six German shorthaired pointer dogs (two females, four males) with exfoliative cutaneous lupus erythematosus (ECLE) were studied in a controlled setting until disease progression necessitated euthanasia. During investigations into the heredity of disease, five dogs received immunomodulatory drugs to alleviate clinical signs (lameness, erythema, scaling, erosions/ulcers). One dog served as a control and received only baths and oral fatty acids. Four dogs received cyclosporin (5–10 mg/kg once daily) for 4.5 months to 2 years. Cyclosporin decreased erythema and arthralgia, but did not halt worsening of lesions. Three dogs received hydroxychloroquine (5–10 mg/kg once daily) for 8 weeks, 7 months, and 9 months, respectively, with no side effects. Hydroxychloroquine appeared to slow clinical progression in two dogs on extended treatment and normalized globulin levels in all three dogs while receiving the drug. Four dogs, including the control dog, were euthanized between 1 and 4.5 years of age. Two remaining male dogs received a tumour necrosis factor (TNF)- α antagonist, adalimumab, at 0.5 mg/kg every 2 weeks for 8 weeks then weekly for 8 weeks. Serum TNF- α levels were not significantly altered nor were quantifiable changes seen in skin lesions or lameness. Subsequently, the dogs were maintained on hydroxychloroquine for another year. This is the first study to evaluate the use of a TNF- α inhibitor for canine lupus and the first to address the safety of long-term administration of hydroxychloroquine, albeit in a small number of dogs. The study documents the progression of ECLE and generally poor response to therapy.

Introduction

In domestic animals, lupoid skin disorders are most commonly diagnosed in the dog.^{1–11} In general, variants of cutaneous lupus erythematosus (CLE) in the dog are skin-localized conditions, and well-described diseases are specific to a few breeds. In contrast, the major clinical signs of dogs with systemic lupus erythematosus (SLE) are nonerosive polyarthritis,

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Conflict of Interest

None.

fever and renal disorders.¹¹ Although a variety of skin lesions may be found in dogs suffering from SLE and cutaneous lesions have been considered a major clinical sign of SLE, referenced citations of canine SLE with lupus-specific dermatopathological features are rare in the veterinary literature.^{3,7,8} Progression from chronic CLE to SLE has not been documented in the dog, but does occur in about 10% of human patients with CLE.¹²⁻¹⁴ A unique and severe form of CLE is seen as a hereditary condition in German shorthaired pointer dogs (GSP).^{1,3,9-11} At less than a year of age, the dogs develop widespread scaling and alopecia with gradual depigmentation and erosions/ulcers; this has been termed 'exfoliative cutaneous lupus erythematosus of German short haired Pointers' (ECLE). The dogs have progressive lethargy and lameness as well as haematological abnormalities. The histological features are lupus-specific; namely, lymphocytic interface dermatitis with marked hyperkeratosis.^{1,3,7-9} Dogs with ECLE are typically euthanized by a few years of age. The natural progression of disease in the aged dog has not been well studied. However, the clinical and histological features in GSPs with ECLE mirror those seen in humans with CLE suggesting the same underlying molecular mechanism.

Treatment of CLE in both people and canine patients involves a balance between desired drug effects (i.e. alleviation of clinical signs) and drug toxicity.^{1,8,12,13,15} Although treatment of ECLE in GSP dogs is largely palliative, periods of remission have been achieved with medical therapy (prednisolone, azathioprine, keratolytic and keratoplastic topicals).^{1,9,10} In a recent study of 25 dogs,¹ the two dogs reported in remission were subsequently euthanized due to disease progression or adverse effects associated with medication (S. Bryden, personal communication). Ciclosporin has been used successfully for the treatment of vesicular CLE in a rough collie dog;¹⁶ and interestingly, one of the two ECLE dogs cited in remission received this drug in combination with other therapeutics.¹

While performing genomic studies to investigate the molecular basis of ECLE in a small group of GSP dogs, the authors herein were challenged with the task of providing comfort as the disease naturally progressed. A controlled environment presented a unique opportunity to investigate new therapeutics and to document treatment response. The following immunomodulatory drugs were evaluated in a sequential manner: ciclosporin (modified), hydroxychloroquine sulphate, and a tumour necrosis factor (TNF)-receptor antagonist (adalimumab). The first two drugs (ciclosporin and hydroxychloroquine) were administered in an attempt to ameliorate clinical signs; whereas adalimumab was given after the authors' experience with the prior drugs and designed as a controlled treatment trial. The objective of this paper was to report the clinical response to the three immunomodulatory drugs and to document the features and progression of ECLE in German shorthaired pointers.

Materials and methods

All dogs were cared for according to the principles outlined in the NIH Guide for the Care and Use of Laboratory Animals and in the International Guiding Principles for Biomedical Research Involving Animals. The youngest dog (Dog 3) was reared in a private home.

Animals

Six GSP dogs with exfoliative CLE were donated over a 3 -year period to the Section of Medical Genetics at the University of Pennsylvania, School of Veterinary Medicine. The dogs consisted of two females (Dogs 1 and 2) and four males (Dogs 3–6) which were donated at ages 15 weeks, 8 months, 8 weeks, 12 months, 17 months and 27 months respectively. At the time of donation, physical examinations, complete blood count (CBC), serum biochemistry profiles, and anti-nuclear antibody (ANA) titres were performed on all dogs. ANA titres were determined by immunofluorescent anti-body (IFA titres in a commercial laboratory (Antech Diagnostics, Inc., Pottstown, PA, USA) with titres less than

1:25 being considered negative. All dogs received baths every 4–7 days with a moisturizing shampoo and conditioner (Hydra-Pearls shampoo and cream rinse, Vétoquinol, Buena NJ, USA; or HyLyte™ shampoo and bath oil or cream rinse, IVX Animal Health, St. Joseph, MO, USA). The dogs also received omega-3 fatty acids (3V Caps™; IVX Animal Health) as a mild anti-inflammatory supplement. In all instances where the dogs displayed signs of pain (e.g. nonweight-bearing lameness for more than 12 h, reluctance to rise or large skin wounds), nonsteroidal medications were added for brief periods of time for humane reasons. This included oral carprofen (Rimadyl™; Pfizer Animal Health, New York, NY, USA) at 2.2 mg/kg twice daily, oral deracoxib (Deramaxx™; Novartis Animal Health Inc., Greensboro, NC, USA) at 1–2 mg/kg once daily, or less commonly buprenorphine (Buprenex™; Reckitt Benckiser Pharmaceuticals Inc., Richmond, VA, USA) at 0.01 mg/kg subcutaneously as needed for severe pain. Antibiotics selected on the basis of culture and sensitivity were used to treat secondary bacterial infections. In the event of euthanasia or natural death, a complete post-mortem examination was performed.

Reproductive evaluation

Vaginal smears were obtained weekly from females to assess signs of heat. Once the dogs were determined to be in proestrus, vaginal cytologies were performed every other day until the first day of diestrus as determined by the cytologies. Vaginal cytology samples were stained with a modified Wright-Giemsa stain (Diff-Quik™; Fisher Scientific, Pittsburgh, PA, USA). Semen evaluations (cell counts, morphology and motility) were performed monthly. Semen samples were stained with eosin nigrin stain (Society for Theriogenology, Auburn AL, USA).

Treatment protocols

One male dog (Dog 4) served as a control and received no systemic treatment apart from essential fatty acids and bath therapy, as described above. Not all dogs received each drug as some of the dogs were donated at different time points during the study and euthanized due to the severity of disease before specific drugs became available. Four dogs entering the study (Dogs 1, 2, 3 and 5) were prescribed oral ciclosporin, modified (Atopica™; Novartis Animal Health, Inc.) at 5 mg/kg to a maximum of 10 mg/kg once daily. Ketoconazole (Apotex Corp., Weston, FL, USA) was added at 5–8 mg/kg orally once daily if clinical improvement was not achieved, or if initial improvement was not maintained. When given concomitantly, ketoconazole interferes with the cytochrome P450 metabolism of ciclosporin in the liver and thereby raises serum levels of ciclosporin.¹⁷ The second immunomodulatory drug administered (Dogs 1, 5 and 6) was hydroxychloroquine sulphate (Watson Pharmaceuticals, Corona, CA, USA) at 5–10 mg/kg orally once daily.

Finally, a controlled trial utilized adalimumab (Humira™; Abbott Laboratories, Abbott Park, IL, USA). For adalimumab, safety and tolerance were first evaluated in two clinically normal, age- and gender-matched beagle dogs housed in the same environment as ECLE dogs using the same dosing protocol. Adalimumab was administered to ECLE Dogs 5 and 6 at 0.5 mg/kg by subcutaneous (s.c.) injection, every 14 days for 8 weeks, then every 7 days for 8 weeks. A washout period of 4 weeks was performed between hydroxychloroquine and adalimumab administration.

Clinical and laboratory analyses

The five dogs housed at the University of Pennsylvania (Dogs 1, 2, 4, 5 and 6) were observed at least three times daily by the same veterinary technicians, and at least once daily by an attending veterinarian. Lameness was evaluated by a board-certified veterinary orthopaedic surgeon and a board-certified veterinary neurologist. CBCs and serum biochemistry screens were performed when treatment protocols were changed, when clinical

signs worsened and during prolonged stabilization of disease to assess overall health. Increased frequency of sampling was performed for drugs with unknown canine safety (weekly during hydroxychloroquine and prior to each adalimumab injection). Urinalyses were also performed before administering a new drug, at least once during and at the end of each treatment protocol with the exception of the adalimumab trial at which time samples were examined monthly. Two 6 mm skin biopsy samples were obtained using local anaesthesia from the lateral or caudodorsal thorax of each dog at the time of donation, prior to the beginning of each new therapy, 1 month after beginning therapy, at least at one time point during therapy and at the end of treatment. The site was chosen due to ease of local anaesthesia, and because the area is consistently affected. Photographs of lesions were taken at the time of admission, intermittently during ciclosporin treatment, monthly during hydroxychloroquine and weekly during the adalimumab treatment and at euthanasia.

Because of the potential cardiac side effects, an electrocardiogram (ECG) was performed prior to treatment with hydroxychloroquine (Dogs 1, 5 and 6), 2 weeks (Dogs 1, 5 and 6), and 3 months (Dogs 5 and 6) after the treatment was begun. The final ECG was performed at the end of treatment (Dogs 5 and 6). The ECGs were reviewed by a board-certified veterinary cardiologist.

Serum ciclosporin concentrations were determined using a commercially available kit according to the manufacturer's directions (Fluorescence Polarization Immunoassay; Abbott Laboratories) every 2–4 months 2 h post-administration of the drug. Hydroxychloroquine serum levels were determined by ELISA (NMS Labs, Philadelphia, PA, USA) 4 h after the morning dose at the end of the study. For adalimumab, blood was obtained immediately prior to giving the very first injection for assessment of serum TNF- α concentrations, and this was repeated every 2 weeks immediately prior to giving each subsequent injection. The TNF- α was measured with a commercially available kit (Quantikine Canine TNF-/TNFSF1A Immunoassay; R&D Systems, Minneapolis, MN, USA). All samples were assayed in duplicate without modification according to the manufacturer's recommendations.

For adalimumab, activity monitor collars were used for a quantitative measure of a potential beneficial response to treatment.¹⁸ The activity monitoring collars were placed on both dogs (Dogs 5 and 6) beginning 2 weeks before the drug trial, the hypothesis being that the better the treatment outcome, the more active and playful the dogs would be. The Actical Activity Monitor™ (AAM; Mini Mitter, Bend, OR, USA) is an omnidirectional accelerometer-based device that measures continuously the intensity, frequency and duration of movement. Output from the AAM is highly correlated with distance travelled, time spent moving and mobility in dogs. The watch-sized monitor was attached to the collar of each dog and positioned ventrally on the neck. The accelerometer epoch was set at 1 min. The AAM can record continuously for 44 days at a 1-min epoch, so the monitor was removed and data were downloaded every 6 weeks. The monitor was then replaced again on that same day. Level and intensity of movement was registered as activity counts, averaged for each 1 min of data collection, downloaded to a computer, and the data analysed using Excel (Microsoft Office, Redmond, WA, USA).

The mean activity values were first compared between both dogs to determine if there were overall differences between their activity levels. Measurements were compared looking at the actual minute data and the means calculated for hours, days, weeks and months. Thereafter, the data derived from the nontreatment period to the treatment period were compared for each dog. Finally, the mean activity levels from both dogs taken together were compared as described. The chi-square and Student's *t*-test were used to statistically evaluate the data.

Results

Summary of clinical features

Skin—All dogs had gross lesions typical of ECLE. The first manifestation of skin disease was adherent scale, followed by erythema with or without pigment loss, erosions and or ulcers with crusted exudate, and progressive thinning of the haircoat to areas of almost complete alopecia (Figure 1a,b). The epidermis along the dorsum was thinned and fragile. The dermis became slightly firm in the older males (Dogs 5 and 6) between 3 and 4 years of age. This was noticeable during skin biopsy sampling. Lesions characteristically affected the head (particularly the dorsal muzzle and pinnae) followed by the dorsal trunk, tail and scrotum. The neck area was uniformly spared. Areas of hair loss never developed new hair growth. Secondary bacterial infections (ear and skin) were common (otitis externa – Dogs 1, 5, 6; superficial pyoderma – all dogs), and some dogs developed acral pruritic nodules (Dogs 1, 5 and 6). Three of the male dogs (Dog 4 – untreated, Dogs 5 and 6) had milder lesions than the females and were older when the lesions progressed. All male dogs developed erosions or ulcers with thick scale and crusts on the scrotum. All dogs had generalized peripheral lymphadenopathy. The skin lesions in two older males (Dogs 5 and 6) never reached the severity of those in Dogs 1–3.

Limbs and joints—Each dog displayed intermittent lameness. The lameness shifted from leg to leg and was best observed within the first 10 min of exercise after which the dogs began to bear more weight on the affected limb. The dogs also developed a hunched stance characterized by a slight dorsal curvature of the spine and stiffness in the hind limbs (Figure 2). Additional diagnostic tests (joints aspirates, pelvic and peripheral limb radiographs, and myelogram) were performed in Dog 1 to further investigate the cause of the lameness and conformation. No abnormalities were found. Dog 3 also had normal pelvic radiographs.

Reproductive aspects—All dogs became infertile including Dog 4 (control, untreated male). All male dogs developed oligospermia as determined by semen evaluations. The oligospermia progressed to azoospermia. A testicular biopsy in Dog 4 (control, untreated) revealed spermatogenic arrest with spermatid giant cells. Dogs 1 and 2 had a single oestrous cycle and irregular cycles respectively.

Histopathology—All dogs had an interface dermatitis characterized by a sparse to mild lymphocytic superficial dermal inflammatory infiltrate, basal keratinocyte vacuolar change with individual basal keratinocyte necrosis, dermoepidermal clefting and pigmentary incontinence (Figure 3). In Dog 3, the interface reaction was identified at the first skin biopsy (12 weeks of age) and progressed to typical changes by 16 weeks (Figure 3a–c). A similar change affected follicular outer root sheaths and was sometimes associated with the loss of sebaceous gland lobules. The histological lesions progressed to marked follicular atrophy and loss and increased dermal connective tissue regardless of the treatment protocol.

Haematology and biochemistry—Baseline lymphocyte counts in three dogs (Dogs 1, 5 and 6) were below normal 50% of the time and ranged between 0.21 and $1.42 \times 10^3/\mu\text{L}$ (normal 0.9 – $5.5 \times 10^3/\mu\text{L}$). Serum globulins were above normal in Dogs 1, 3, 5 and 6 at least 50% of the time (range 4.1 – 5.0 g/dL; normal 2.4 – 4.0 g/dL). Four dogs had minimal to mild thrombocytopaenia (Dogs 1, 3, 4 and 6) at one or more time points on an instrument count (averaged 130 – $160 \times 10^3/\mu\text{L}$; normal range 177 – 398×10^3). In Dog 6, the platelet count was consistently low (96 – 159×10^3) regardless of treatment. In all instances, a manual platelet count revealed normal to slightly decreased platelets. Antinuclear antibodies were not detected in any of the dogs at any given time and other than serum globulins mentioned above, serum biochemistry screens remained within normal ranges.

Post-mortem examinations—Post-mortem examination of Dogs 1, 2, 3 and 4 revealed reactive lymphoid hyperplasia in peripheral lymph nodes, mild to moderate lymphoplasmacytic and eosinophilic enteritis, and colitis which was severe in Dog 3. Dog 4 (untreated control) had localized demodicosis on the scrotum and Dog 1 had a focal viral papilloma on the face. Gross and histological examination of clinically affected joints was normal. No abnormalities were found on histological evaluation of the spinal cord and peripheral nerves in Dog 3.

Treatment results

Ciclosporin—Ciclosporin treatment was started in Dog 1 at 1 year of age and lasted 2 years. A marked improvement was seen after 1 week of treatment (increased activity and appetite, decreased erythema and lameness) (see Table 1). The improvement persisted for a few months. Brief periods of drug withdrawal resulted in increased lameness. The skin lesions waxed and waned but slowly worsened despite dose adjustments (i.e. increasing the dose of ciclosporin, then adding ketoconazole), and the addition of prednisone during a 10-week period of lesion progression.

In Dog 2, ciclosporin treatment was started at 7 months of age. After 10 days, the dog had improved ambulation with decreased erythema and increased activity level such that all pain medications were stopped. The skin lesions slowly progressed but the dog remained comfortable for approximately 6 months. At 13 months of age, the lameness markedly worsened despite the addition of pain management drugs. The skin lesions became more erosive and the dog developed meticillin-resistant *Staphylococcus pseudintermedius* pyoderma, which did not respond to culture-based antimicrobial treatment. The dog was euthanized at 14 months of age.

Dog 3 had developed lameness, variable diarrhoea and skin lesions by 10.5 months of age. Within 10 days of starting ciclosporin, the dog displayed a marked improvement in activity level, demeanour and ambulation (started climbing stairs; acting playful). Six weeks later, the dog worsened and lameness and skin lesions progressed despite increasing the ciclosporin dose and adding ketoconazole to the drug regimen. The skin lesions quickly progressed with lameness and a hunch stance becoming severe and nonresponsive to non-steroidal anti-inflammatory drugs. The dog was euthanized at 1.5 years of age. Dog 5 was started on ciclosporin at 17 months of age and received the drug for 5 months. A decrease in lameness and slight decrease in erythema was observed while on medication. Skin lesions slowly progressed but never reached the severity seen in the other treated dogs. Systemic absorption was confirmed by serum in analysis in all dogs such that the lack of response was not due to failure of absorption. Dog 6 was not treated with ciclosporin as the dog was donated at a later time, when other therapies were being evaluated.

Hydroxychloroquine—Dog 1 received hydroxychloroquine sulphate (5 mg/kg orally once a day) at 3 years of age for 8 weeks, following progressive worsening of skin lesions despite extended treatment with ciclosporin. The skin lesions remained the same while the dog received hydroxychloroquine. The dog was removed from the study for reasons unrelated to ECLE. Dogs 5 and 6 received hydroxychloroquine sulphate at 8–10 mg/kg orally once daily for 7 and 9 months respectively. The skin lesions and intermittent lameness did not resolve but did not progress in severity during this time. Hydroxychloroquine serum concentrations were within a therapeutic range of that expected in a treated person (Dog 5: 0.56 µg/dL; Dog 6: 0.87 µg/mL; therapeutic range 0.1–1 µg/mL given by NMS Labs) and decreased to undetectable concentrations 2 weeks after cessation of treatment. ECG and ocular examinations were normal, and no adverse changes were seen in the CBC and chemistry screens compared to baseline. Each dog had persistently high normal or elevated

globulin levels at the start of therapy (4.5, 5 and 4.3 mg/dL respectively; reference range 2.4–4 mg/mL), which normalized 6 weeks (Dogs 5 and 6) or 7 weeks (Dog 1) after treatment began and increased back to the elevated baseline within 6–10 weeks after cessation of treatment.

Adalimumab—Prior to administration to ECLE dogs, adalimumab given to the two normal beagle dogs did not result in any abnormalities. Serum biochemical, haematological and urinalysis values stayed within reference ranges, and no changes were seen on physical examination. Dogs 5 and 6 completed the 16-week treatment course with no adverse effects. Each dog displayed a subjective improvement (improved demeanour, slight decrease in lameness) after 2 and 3 weeks of treatment, respectively, with decreased diameter of scrotal ulcers. Following the initial month of treatment, the lesions and lameness returned to baseline. While it appeared that lesions worsened after discontinuation of treatment, the change could not be quantified. The activity monitors revealed a trend towards increased activity after treatment began in Dog 5 but this was not significant ($P < 0.05$). Serum TNF- α concentrations were determined in a normal control dog (unrelated beagle mix) and in Dogs 5 and 6 before treatment and during adalimumab therapy (normal <7.8 pg/mL). Before adalimumab administration, serum concentrations of TNF- α in dogs 5 and 6 ranged from 7.0 to 12.3 pg/mL (mean 9.98 ± 1.62 pg/mL) based on three samples per dog obtained during a time period when the dogs were not receiving any medications. Throughout the treatment trial with adalimumab, the serum TNF- α concentrations fluctuated from <0.1 to 13.2 pg/mL with no apparent pattern in relation to the administration of the drug (Figure 4).

Discussion

This study confirmed that ECLE of GSP dogs will progress despite any form of therapy and even without the influence of sunlight, or environmental factors known to trigger or exacerbate some forms of lupus.^{12,14} Besides evaluating immunomodulatory drugs in a controlled setting, this study is the first to document the use and safety of hydroxychloroquine sulphate in veterinary medicine. The study was part of a larger investigation into the genetic basis of the disease and developed over time as the authors strove to maintain humane comfort for the dogs. The oldest dogs (5 and 6) were still alive and comfortable at the time this manuscript was written (4 and 5 years of age respectively) and maintained only with oral hydroxychloroquine and intermittent periods of anti-inflammatory doses of prednisolone.

In people, all forms of lupus are thought to develop from similar immune mechanisms regardless of the clinical manifestation.¹² CLE refers to the skin-specific effects of LE whether or not there is systemic involvement (i.e. joints, kidney, blood, etc.). In general, cutaneous lupus disorders have in common a constellation of 'lupus specific' histopathological features which may include the following: hyperkeratosis, epidermal atrophy, basal cell vacuolar degeneration, basement membrane thickening and mononuclear cell infiltration at the dermoepidermal interface. While skin disorders do commonly occur in lupus patients, they may not be specific for lupus and can be found with other diseases (e.g. vasculitis, telogen effluvium, leg ulcers, calcinosis cutis, etc.). More importantly, it is the pattern and extent of lupus-specific skin lesions that help to predict the clinical course of the patients' disease.^{12,19,20} In humans, CLE is sub-grouped into three major categories: chronic CLE (localized and generalized discoid LE), subacute LE and acute LE. Chronic CLE is more likely to remain skin-localized than acute or subacute CLE. However, patients with chronic CLE who have generalized skin involvement (i.e. disseminated discoid LE) do have increased risk of developing systemic manifestations of LE.^{12,14,19-21} The diagnosis of SLE in human and veterinary patients is based on fulfilment of four of 11 criteria established by

the American College of Rheumatology with virtually all cases having a positive ANA titre.²⁰

The disease in the German shorthaired pointer dog is clearly a disseminated form of cutaneous lupus. In our group of dogs, the cutaneous lesions become readily apparent between 4 and 6 months of age. As the skin lesions progressed (typically from the face to the trunk and extremities), the dogs then developed arthralgia, manifested as shifting leg lameness and a tucked-up or hunched stance, along with mild haematological changes (lymphopaenia, thrombocytopaenia). The disease mimics the progression of disseminated discoid lupus to SLE in humans; but to date, consistent antinuclear antibodies were not documented in our dogs or in prior studies.^{1,9,10} From this data, we surmise that clues to the pathogenesis of ECLE in dogs may have far-reaching effects to the lupus disorders in other species and most importantly in humans.

The clinical signs and histological features in this group of GSP dogs were similar to those cited in previous studies.^{1,3,9,10} There was an inherent phenotypic variation in disease progression and severity, but the clinical lesions were identical in all dogs. At times, the arthralgia appeared to be more physically debilitating than the erosive skin lesions. The lameness shifted from leg to leg and was most pronounced during the first 10 min of exercise after which the dogs began to bear more weight on the affected limb. The hunched stance was a progressive change that remained constant once established. When severe, the dogs had difficulty rising or displayed an unwillingness to climb stairs. The lameness worsened with age in all dogs. Extensive diagnostics performed to determine the source of the arthralgia were negative and these included complete orthopaedic and neurological examinations, radiographs, joint aspirates, myelogram (one dog), and detailed post-mortem examination of joint and nervous tissues. Similar findings were cited in a recent retrospective analysis.¹ In humans, arthralgia occurs in about 30% of humans within subacute CLE.^{13,22} In the absence of erosive joint lesions, the cause of arthralgia in humans is not clear but may be cytokine induced.²²

Not previously reported was the progressive infertility in all dogs. The main features were low sperm counts and abnormal oestrous cycles. In the males, stress from the disease itself combined with localized scrotal hyperthermia may have interfered with spermatogenesis. It is very difficult to associate episodes of severe scrotal inflammation with poor semen quality because of the long lag phase until sperm cells are renewed (60–75 days).²³ Low sperm counts have been reported in men with SLE particularly those receiving cytotoxic therapy.²⁴ Furthermore, an increased frequency of infertility has been seen in women with SLE.²⁵ It is important to note that Dog 4 received no systemic drugs other than fatty acids, and had gradual oligospermia, which also progressed to azoospermia.

In humans, disseminated CLE without systemic signs is routinely treated with antimalarial drugs. Hydroxychloroquine sulphate is the antimalarial of choice as it has fewer side effects than quinidine or chloroquine.^{26–28} Hydroxychloroquine is initially prescribed as monotherapy but due to a unique mechanism of action, it can be used in combination with other immunomodulatory drugs.^{26–28} When given orally to humans, the antimalarials have a slow onset of action (6–8 weeks) and function by down-regulating the immune response. Specifically, antimalarials increase the pH in cellular compartments and thereby interfere with the formation of peptide-MHC protein complexes which are needed to stimulate CD4⁺ T cells.²⁶ Serious side effects of hydroxychloroquine in humans are uncommon. An irreversible retinopathy is rare, and risk factors include long-term therapy (usually >5 years) at high doses, advanced age and renal insufficiency. Other adverse effects are reversible corneal lipid deposits, irritability, hyperactivity, seizures, vomiting/diarrhoea, reversible

haematological abnormalities and cutaneous eruptions. Cardiotoxicity is also rare and more likely to occur in patients with SLE.^{13,14,26-28}

The administration of hydroxychloroquine to the ECLE dogs was conducted with scrutiny. A canine study performed more than three decades ago cited cardiotoxic effects of antimalarials in anaesthetized dogs.²⁹ Specifically, dynamic cardiac changes were seen after repeated intravenous administration of high doses of hydroxychloroquine and chloroquine. No ante- or post-mortem tissue examinations were performed and there was no mention of the outcome of the dogs' long-term health including if the dogs had died during the experiment. It remains unclear if a lasting effect (e.g. structural myocardial damage) would have ensued. The study did show that chloroquine had a greater cardiodepressive effect than hydroxychloroquine.²⁹

The ECLE dogs treated with hydroxychloroquine showed no adverse clinical signs. Electrocardiograms and ophthalmic examinations were normal throughout the trial. Dogs 5 and 6 were given a higher dose than Dog 1 and treated for a much longer period of time. The dose (approximately 10 mg/kg) is higher on a per weight basis than that recommended for a human (6.5 mg/kg maximum dose based on lean body weight).²⁸ The hydroxychloroquine serum concentrations, measured at study terminus, were within the therapeutic range for a human.³⁰ In humans, the drug reaches peak concentrations at about 8 h and a state of equilibrium after several weeks.²⁸ In Dogs 5 and 6, the disease appeared to stabilize and significant disease progression was not seen during the 7 and 9 months of treatment. Interestingly, all dogs (1, 5 and 6) developed normal serum globulin concentrations during treatment with hydroxychloroquine after having been consistently elevated irrespective of other therapies. The mechanism causing this change is unclear, but it is possible that hydroxychloroquine either interfered with self-antigen presentation and immunoglobulin production or that it had an overall effect of decreasing inflammation. Additional testing and pharmacokinetic studies should be pursued to confirm the safety of the drug in a larger number of dogs and to determine the most appropriate canine dose.

Ciclosporin has proved to be safe and effective for the treatment of dogs with refractory atopic dermatitis and other skin conditions.^{17,31-34} The ciclosporin treatment began with enthusiasm as the first dog treated (Dog 1) had a dramatic response within 10–14 days. The second dog (Dog 3) was suffering from marked lameness when the drug was started. Within 10 days, all pain medications were withdrawn; the dog started climbing stairs, which had been previously avoided. The authors also treated a client-owned dog (not cited in the study) that showed marked improvement (decreased erythema) but was then lost to follow-up. Unfortunately, ciclosporin did not halt disease progression in any dog; and as time passed, it was difficult to assess what effect, if any, the drug had on the disease. In fact, Dog 1 received the drug for 2 years and despite dose adjustments (up to 10 mg/kg), with confirmed systemic absorption, the drug never produced the same effect seen in the initial response. This effect, partial remission then failure to respond, has been reported with both azathioprine and prednisone in ECLE.¹

As a potent inhibitor of T-cell activation, which it achieves through inhibition of protein translation, ciclosporin may be effective in decreasing T-cell-dependent clinical effects of cutaneous lupus.³⁵ More specifically, the individual basal cell necrosis has been hypothesized to be a specific attack on keratinocytes by activated T cytotoxic cells.¹ Indeed, the positive effects seen in a few dogs may have been related to impairment of cell-mediated immunity, but likely by blocking the transcription T-cell-dependent cytokines via inhibition of calcineurin. Ciclosporin is an immunomodulatory rather than a cytotoxic drug.³⁶ The effect was likely similar to the decrease in pruritus and inflammation observed in atopic dogs treated with ciclosporin, rather than impairment of T-cell cytotoxicity.^{17,31-34}

However, the failure to sustain the response and failure to substantially improve cutaneous lesions likely reflects the humoral basis of the disease pathogenesis. Immunoglobulin deposition, particularly IgG, has been documented in the basement membrane of the epidermis and adnexa in ECLE.¹

Adalimumab, a TNF- α antagonist, was administered to Dogs 5 and 6 with strict control of outside parameters. To the authors' knowledge, this class of drugs has not been used in veterinary medicine. TNF- α antagonists have shown great promise in treating an array of noninfectious inflammatory and autoimmune diseases in humans [e.g. rheumatoid arthritis (RA), pemphigus complex diseases, lupus erythematosus, Crohn's disease and psoriasis/psoriatic arthritis].³⁷⁻⁴¹ TNF- α is a pro-inflammatory cytokine involved in a wide range of physiological processes, including mediation of infections and promotion of inflammatory diseases. TNF- α antagonists have shown superior disease control compared to 'standard' drug therapies, although a primary adverse effect may be increased risk of infection (approximately 2 $\times$ nontreated controls).^{42,43} For autoimmune skin diseases of humans, this class has been used to rescue recalcitrant cases and to prolong disease-free intervals. These drugs also increase the ability to control the disease with low-dose glucocorticoids after a total of four to six injections.^{38,40,44} However, all TNF- α inhibitors can potentially exacerbate clinical signs of SLE and increase autoantibody levels directed against double-stranded DNA and cardiolipin.⁴⁵⁻⁴⁷ Although approved by the U.S. Food and Drug Administration only for the treatment of RA, reports of the successful use of these compounds in treating a variety of autoimmune skin disorders, including LE, have been published.^{37-44,48} As the ECLE dogs were affected by both cutaneous lupus and arthralgia, efficacy of a TNF- α inhibitor was postulated.

The parameters for evaluation during the adalimumab trial were strictly defined. Activity collars, photographic documentation of skin lesions and histopathology were used in an attempt to provide objective measurements of treatment response. After 2 weeks, the drug did appear to have a mild ameliorating effect but the change could not be objectively documented. There was also no correlation with circulating TNF- α levels. As the product is a human monoclonal antibody, it is possible that binding did not occur *in vivo*, although binding to canine TNF- α has been demonstrated *in vitro* (H. Koprowski, Thomas Jefferson University, unpublished data). It is more likely that the dogs mounted an antibody response against the molecule, which inactivated it. Further investigation should be considered, as no adverse effects were noted in this small population of dogs. Using our dosing regimen and based upon current market prices, adalimumab therapy is comparable in cost to name-brand ciclosporin A modified (AtopicaTM).

The trials herein document the inherent progressive nature of ECLE despite extended course immunomodulatory therapy. Hydroxychloroquine can be used safely in dogs and is a drug that warrants further investigation.

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References

1. Bryden SL, White SD, Dunston SM, et al. Clinical, histopathological and immunological characteristics of exfoliative cutaneous lupus erythematosus in 25 German short-haired pointers. *Veterinary Dermatology*. 2005; 16:239–52. [PubMed: 16101795]
2. Gross, TL.; Ihrke, PJ. Ulcerative dermatosis of Shetland Sheepdog and Collie. In: Bonagura, JD.; Kirk, KW., editors. *Current Veterinary Therapy XII: Small Animal Practice*. W.B. Saunders; Philadelphia, PA: 1995. p. 639-70.
3. Gross, TL.; Ihrke, PJ.; Walder, EJ., et al. *Skin Diseases of the Dog and Cat. Clinical and Histopathologic Diagnosis*. 2nd edn.. Blackwell Science, Ltd; Ames, IA: 2005. Interface diseases of the dermal-epidermal junction; p. 49-74.
4. Jackson HA. Eleven cases of vesicular cutaneous lupus erythematosus in Shetland sheepdogs and rough collies: clinical management and prognosis. *Veterinary Dermatology*. 2004; 15:37–41. [PubMed: 14989704]
5. Jackson HA, Olivry T. Ulcerative dermatosis of the Shetland sheepdog and rough collie dog may represent a novel vesicular variant of cutaneous lupus erythematosus. *Veterinary Dermatology*. 2001; 12:19–27. [PubMed: 11301535]
6. Olivry T, Savary KC, Murphy KM, et al. Bullous systemic lupus erythematosus (type I) in a dog. *Veterinary Record*. 1999; 145:165–9. [PubMed: 10466774]
7. Scott DW, Walton DK, Manning TO, et al. Canine systemic lupus I. Systemic lupus erythematosus. *Journal of the American Animal Hospital Association*. 1983; 19:461–79.
8. Scott, DW.; Griffin, CE. *Muller and Kirk's Small Animal Dermatology*. 6th edn.. W.B. Saunders; Philadelphia, PA: 2001. Immune-mediated disorders; p. 701-17.
9. Vroom MW, Theaker MJ, Rest JR, et al. Lupoid dermatosis in five German short-haired pointers. *Veterinary Dermatology*. 1995; 6:93–8.
10. Theaker AJ, Rest JR. Lupoid dermatosis in a German short haired pointer. *Veterinary Record*. 1992; 131:495. [PubMed: 1471334]
11. Stone, M. Systemic lupus erythematosus. In: Ettinger, SJ.; Feldman, EC., editors. *Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat*. 6th edn.. Elsevier/Saunders; St. Louis, MO: 2005. p. 1952-7.
12. Costner, MI.; Sontheimer, RD. Lupus erythematosus. In: Freedberg, IM.; Eisen, AZ.; Wolff, K., et al., editors. *Fitzpatrick's Dermatology in General Medicine*. 6th edn.. McGraw-Hill; New York: 2003. p. 1677-93.
13. Patel P, Werth V. Cutaneous lupus erythematosus: a review. *Dermatologic Clinics*. 2002; 20:373–85. [PubMed: 12170873]
14. Werth VP. Cutaneous lupus: insights into pathogenesis and disease classification. *Bulletin of the NYU Hospital for Joint Diseases*. 2007; 65:200–4. [PubMed: 17922670]
15. Werth V. Current treatment of cutaneous lupus erythematosus. *Dermatology Online Journal*. 2001; 7:2. [PubMed: 11328623]
16. Font A, Bardagi M, Mascort J, et al. Treatment with oral cyclosporin A of a case of vesicular cutaneous lupus erythematosus in a rough collie. *Veterinary Dermatology*. 2006; 17:440–2. [PubMed: 17083576]
17. Guaguere E, Steffan J, Olivry T. Cyclosporin A: a new drug in the field of canine dermatology. *Veterinary Dermatology*. 2004; 15:61–74. [PubMed: 15030555]
18. Hansen B, Lascelles B, Keene B, et al. Evaluation of an accelerometer for at-home monitoring of spontaneous activity in dogs. *American Journal of Veterinary Research*. 2007; 68:468–75. [PubMed: 17472445]
19. Sontheimer RD. The lexicon of cutaneous lupus erythematosus – a review and personal perspective on the nomenclature and classification of the cutaneous manifestations of lupus erythematosus. *Lupus*. 1997; 6:84–95. [PubMed: 9061656]

20. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis and Rheumatism*. 1982; 25:1271–7. [PubMed: 7138600]
21. Lin JH, Dutz JP, Sontheimer RD, et al. Pathophysiology of cutaneous lupus erythematosus. *Clinical Reviews in Allergy and Immunology*. 2007; 33:85–106. [PubMed: 18094949]
22. Rothfield N, Sontheimer RD, Bernstein M. Lupus erythematosus: systemic and cutaneous manifestations. *Clinical Dermatology*. 2006; 24:348–62.
23. Johnston, SD.; Root Kustritz, MV.; Olson, PS. *Canine and Feline Theriogenology*. 1st edn.. W.B. Saunders; Philadelphia, PA: 2001. Sexual differentiation and normal anatomy of the dog; p. 275-86.
24. Soares PM, Borba EF, Bonfa E, et al. Gonad evaluation in male systemic lupus erythematosus. *Arthritis and Rheumatism*. 2007; 56:2352–61. [PubMed: 17599762]
25. Geva E, Lerner-Geva L, Burke M, et al. Undiagnosed systemic lupus erythematosus in a cohort of infertile women. *American Journal of Reproductive Immunology*. 2004; 51:336–40. [PubMed: 15212668]
26. Kalia S, Dutz JP. New concepts in antimalarial use and mode of action in dermatology. *Dermatologic Therapy*. 2007; 20:160–74. [PubMed: 17970883]
27. Wozniacka A, Carter A, McCauliffe DP. Antimalarials in cutaneous lupus erythematosus: mechanisms of therapeutic benefit. *Lupus*. 2002; 11:71–81. [PubMed: 11958581]
28. Wozniacka A, McCauliffe DP. Optimal use of antimalarials in treating cutaneous lupus erythematosus. *American Journal of Clinical Dermatology*. 2005; 6:1–11. [PubMed: 15675885]
29. Lansimies E, Laaksonen AL, Juva K, et al. Acute cardiotoxicity of chloroquine and hydroxychloroquine in dogs. *Annales Medicinæ Experimentalis et Biologiæ Fenniae*. 1971; 49:45–8. [PubMed: 5576242]
30. Mackenzie AH. Dose refinements in long-term therapy of rheumatoid arthritis with antimalarials. *American Journal of Medicine*. 1983; 75:40–5. [PubMed: 6869410]
31. Olivry T, Rivierre C, Jackson HA, et al. Cyclosporine decreases skin lesions and pruritus in dogs with atopic dermatitis: a blinded randomized prednisolone-controlled trial. *Veterinary Dermatology*. 2002; 13:77–87. [PubMed: 11972891]
32. Olivry T, Steffan J, Fisch RD, et al. Randomized controlled trial of the efficacy of cyclosporine in the treatment of atopic dermatitis in dogs. *Journal of the American Veterinary Medical Association*. 2002; 221:370–7. [PubMed: 12164533]
33. Steffan J, Favrot C, Mueller R. A systematic review and meta-analysis of the efficacy and safety of cyclosporin for the treatment of atopic dermatitis in dogs. *Veterinary Dermatology*. 2006; 17:3–16. [PubMed: 16412115]
34. Steffan J, Parks C, Seewald W. Clinical trial evaluating the efficacy and safety of cyclosporine in dogs with atopic dermatitis. *Journal of the American Veterinary Medical Association*. 2005; 226:1855–63. [PubMed: 15934253]
35. Mascarell L, Truffa-Bachi P. New aspects of cyclosporin a mode of action: from gene silencing to gene up-regulation. *Mini Reviews in Medicinal Chemistry*. 2003; 3:205–14. [PubMed: 12570836]
36. Losa Garcia JE, Mateos Rodriguez F, Jimenez Lopez A, et al. Action of cyclosporin A on mononuclear phagocytes. *Journal of Investigative Allergology and Clinical Immunology*. 1996; 6:222–31.
37. Pardo J, Mercader P, Mahiques L, et al. Infliximab in the management of severe pemphigus vulgaris. *British Journal of Dermatology*. 2005; 153:222–3. [PubMed: 16029365]
38. Jacobi A, Shuler G, Hertl M. Rapid control of therapy-refractory pemphigus vulgaris by treatment with the tumour necrosis factor-alpha inhibitor infliximab. *British Journal of Dermatology*. 2005; 153:448–9. [PubMed: 16086769]
39. Tobin AM, Kirby B. TNF alpha inhibitors in the treatment of psoriasis and psoriatic arthritis. *BioDrugs*. 2005; 19:47–57. [PubMed: 15691217]
40. Sacher C, Rubbert A, Konig C, et al. Treatment of recalcitrant cicatricial pemphigoid with the tumor necrosis factor alpha antagonist etanercept. *Journal of the American Academy of Dermatology*. 2002; 46:113–5. [PubMed: 11756956]
41. Berookhim B, Fischer HD, Weinberg JM. Treatment of recalcitrant pemphigus vulgaris with the tumor necrosis factor alpha antagonist etanercept. *Cutis*. 2004; 74:245–7. [PubMed: 15551718]

42. Rychly DJ, DiPiro JT. Infections associated with tumor necrosis factor-alpha antagonists. *Pharmacotherapy*. 2005; 25:1181–92. [PubMed: 16164393]
43. Scheinfeld N. A comprehensive review and evaluation of the side effects of the tumor necrosis factor alpha blockers etanercept, infliximab and adalimumab. *Journal of Dermatological Treatment*. 2004; 15:280–94. [PubMed: 15370396]
44. Lin MH, Hsu CK, Lee JY. Successful treatment of recalcitrant pemphigus vulgaris and pemphigus vegetans with etanercept and carbon dioxide laser. *Archives of Dermatology*. 2005; 141:680–2. [PubMed: 15967912]
45. Aringer M, Smolen JS. Efficacy and safety of TNF-blocker therapy in systemic lupus erythematosus. *Expert Opinion on Drug Safety*. 2008; 7:411–9. [PubMed: 18613805]
46. Ramos-Casals M, Brito-Zeron P, Munoz S, et al. Autoimmune diseases induced by TNF-targeted therapies: analysis of 233 cases. *Medicine (Baltimore)*. 2007; 86:242–51. [PubMed: 17632266]
47. Ramos-Casals M, Brito-Zeron P, Soto MJ, et al. Autoimmune diseases induced by TNF-targeted therapies. *Best Practice & Residential Clinical Rheumatology*. 2008; 22:847–61.
48. Aringer M, Graninger WB, Steiner G, et al. Safety and efficacy of tumor necrosis factor alpha blockade in systemic lupus erythematosus: an open-label study. *Arthritis and Rheumatism*. 2004; 50:3161–9. [PubMed: 15476222]

Figure 1.

Photograph: progressive skin changes in Dog 1: (a) at 9 months of age the dorsal muzzle has mild scaling, alopecia and few adherent crusts. Two years later (b), the hair loss is more severe with ulcer/erosions and marked depigmentation.

Figure 2.
Photograph: Dog 1. Typical 'hunched stance' with dorsal curvature of the spine and limbs slightly tucked under the body.

Figure 3.

Photomicrographs: Sequential skin biopsies from Dog 3. At 3 months of age (a), few lymphocytes infiltrate the basal cell layer. A mild vacuolar change is evident in few keratinocytes with rare individually necrotic basal cells (arrows). At 4 months of age (b), the corneal layer is expanded by marked laminar orthokeratotic hyperkeratosis. The epidermis is moderately acanthotic with similar mild changes in the basal cell layer. At 10 months (c), the dermoepidermal junction is obscured by a more pronounced vacuolar change and lymphocytic infiltrate. Some individually necrotic keratinocytes (arrows) are located in the spinous layer. H&E, 20 $\times$, Bar = 50 μ m.

Figure 4.

Graph: Serum TNF- α concentrations of adalimumab in one normal and two affected dogs before and after 8 weeks of biweekly s.c. injections and 4 weeks of weekly s.c. injections. There are no significant differences between any data points.

Table 1
Summary of sequential drug therapy and treatment outcome in ECLE GSP dogs

Sequential treatments	Dog	Duration (months)	Initial clinical response	Outcome summary
(1a) Ciclosporin 5–10 mg/kg once daily orally	1	5	Increased activity; decreased erythema/lameness	Disease progression
	2	2	Increased activity; decreased erythema/lameness	Disease progression
	3	2	Increased activity; decreased lameness	Disease progression
	5	5	Slight decrease erythema/lameness	Disease progression
	1	17	Waxing/waning lesions	Disease progression
(1b) Ciclosporin + ketoconazole 5–8 mg/kg once daily orally	2	4	Disease progression	Euthanized (1.2 years)
	3	3	Disease progression	Euthanized (1.5 years)
	1	2	Unchanged	Euthanized (3.25 years)
(2) Hydroxychloroquine 5–10 mg/kg once daily orally	5	7	No progression	Disease stable
	6	9	No progression	Disease stable
	5	2	Slight decrease, scrotal ulcers and lameness	Return to baseline
(3a) Adalimumab 0.5 mg/kg biweekly s.c.	6	2	Slight decrease, scrotal ulcers and lameness	Return to baseline
	5	2	Unchanged	Alive (3.5 years)
	6	2	Unchanged	Alive (4.25 years)

Dog 4 served as a control and received no immunomodulatory drugs.