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Clinical and histopathological features of pemphigus foliaceus with and without eosinophilic infiltrates: a retrospective evaluation of 40 dogs

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Abstract

The importance of cellular infiltrates in tissues has been investigated as a diagnostic tool, mechanism of pathogenesis, and prognostic indicator in certain human diseases. Eosinophils, in particular, have a distinct role in the development of cutaneous lesions in human autoimmune diseases. Identification of an eosinophilic infiltrate can aid the diagnosis of immunobullous disease in the early stages of the disease process. In canine pemphigus foliaceus, eosinophils are present to a variable degree within lesional tissue. This study retrospectively evaluated 40 dogs with pemphigus foliaceus, and examined clinical and histologic features and final outcomes in cases with and without eosinophilic infiltrates. Twenty-five of 40 dogs (63%) had an eosinophilic infiltrate in either the pustules⁄crust, follicular infundibulum or dermis. There was no statistically significant difference in clinical distribution or appearance of dermatological lesions, response to treatment, or disease outcome in dogs with or without an eosinophilic infiltrate. However, dogs with concurrent disease were significantly more likely to have an eosinophilic infiltrate (_P_ = 0.01). Dogs with adverse effects associated with immunosuppressive therapy were significantly more likely to have an eosinophilic infiltrate (_P_ = 0.05). Fifteen of 40 dogs (38%) had a history of allergic disease and a significantly higher proportion of these dogs had an eosinophilic infiltrate (_P_ = 0.04). An eosinophilic infiltrate was found in more than half of the dogs in this study. These findings justify further studies to investigate the role of eosinophils in the pathogenesis, therapy and prognosis in dogs with pemphigus foliaceus.

Introduction

Pemphigus foliaceus (PF) is the most common autoimmune disorder in dogs, and is also the most frequent manifestation of the pemphigus complex in small animals.[1] Since the first report of canine PF in 1977,[2] numerous studies have defined the clinical presentation, histopathological findings, immunopathology, therapeutic regimens and treatment outcomes in domestic animals.[3–9] Classically, the histopathological appearance of PF in dogs is a subcorneal or intragranular pustular dermatitis with pustules containing acantholytic cells and spanning multiple hair follicles.[10,11] Active acantholysis may be seen beneath the pustule. Neutrophils predominate within pustules, but eosinophils can be present to a variable degree.[10]

Although the presence of eosinophils in PF has been reported in dogs, no studies have examined whether this is a result of concurrent disorders, or whether it influences the clinical presentation, therapeutic outcome or prognosis. In contrast, the significance of eosinophil-rich infiltrates has been evaluated in a variety of human diseases. Eosinophilic infiltration in squamous cell carcinoma can be a histopathological criterion in determining stromal invasion and tumour biology.[12–14] Eosinophil-predominant Hodgkin's lymphoma is associated with worse treatment results and a poorer prognosis.[15–17] The presence of eosinophils and neutrophils within the epidermis may be recognized as the first indication of immunobullous and other inflammatory dermatological disorders in people.[18,19] In addition, analyses of chemotactic cytokines in blister fluid and sera have been correlated with the character of the cellular infiltrate in people with autoimmune blistering diseases.[20–22] Such patterns of inflammation and specific types of infiltrating cells can provide valuable clinical, prognostic and diagnostic information to the clinician. The purpose of this study was to retrospectively examine the clinical and histopathological features of 40 dogs with PF, and describe features associated with the presence of an eosinophilic infiltrate.

Materials and methods

Study population and design

The Department of Pathobiology and the Louisiana Animal Disease Diagnostic Laboratory (LADDL), Louisiana State University School of

Veterinary Medicine (LSUSVM), and PATHodgin Veterinary Dermatopathology Service databases between 2002 and 2008 were searched for cases of canine PF. To be included in the study, cases must have been submitted by dermatologists at the LSUSVM, Veterinary Teaching Hospital & Clinics (VTH&C), or by one of two board-certified veterinary dermatologists in private referral practices. Only medical records of cases with complete signalment, history and clinical description were included. In accordance with standards recommended by Olivry,[5] further criteria for inclusion included a definitive diagnosis of pemphigus supported by histopathological findings based on established criteria,[10,11] a negative fungal culture to rule out acantholytic dermatophytosis and classic clinical signs.[1,4] Dogs with concurrent pyoderma were not excluded from the study.

Data collected from the medical records included signalment, history of prior skin disease, clinical signs at the time of presentation, medications used within the 3 months prior to biopsy, concurrent systemic disease, laboratory tests analysed prior to treatment, time from lesion onset to diagnosis, season of diagnosis, dermatological lesions, distribution of disease, initial treatment, adverse effects of initial treatment, maintenance treatment, time to remission, follow-up time and outcome. Final treatment outcomes were recorded as remission (no active pustular disease), death (including euthanasia), improvement or no improvement (active pustular disease). Improvement was defined in accordance with the study by Mueller et al.,[4] as residual erythema, scaling or crust without the need for change in treatment.

Histopathology

The original slides for cases selected for this study were re-evaluated by one pathologist (ECH). Originally, formalin fixed tissues were trimmed for histological processing then embedded in paraffin, sectioned at 3 μm, and stained with haematoxylin and eosin (H&E). Each specimen from each case was examined for the following histological changes typical of PF: acantholytic crust, acantholytic epidermal and infundibular pustules, folliculitis with acantholytic cells, and superficial dermatitis with neutrophils and/or eosinophils, lymphocytes, plasma cells and histiocytes. The specimen containing the best examples of these changes was scored for the following:

1 Estimated percentages of eosinophils and neutrophils/total granulocytes in crust, epidermal and infundibular pustules, infundibular lumens and upper dermis after examining a minimum of ten typical areas. Inflammation due to furunculosis was ignored. Scores were recorded as: DG (crust too degenerate to evaluate), 0 (0–5%), 1 + (6–40%), 2 + (41–60%), 3 + (61–95%), or 4 + (96–100%).

2 Numbers of acantholytic cells and cells undergoing active acantholysis in the most prominent acantholytic areas of the crust, epidermal and infundibular pustules and infundibular lumens. Scores were recorded as: 0 (none seen/10 high power fields [hpf]), 1+ (less than 10 cells/10 hpf), 2+ (11–30 cells/10 hpf), or 3+ (31 or more cells/10 hpf). High power field magnification was 400×.

Statistical analysis

All variables were described and summarized by frequencies for categorical variables, including ordinal scores, and quartiles for numeric variables. For analysis, categories of many variables were collapsed to increase strata density. To explore the associations of an eosinophilic infiltrate with clinical signs and disease outcome, any dog with an eosinophilic infiltrate in either the pustules/crust, follicles or dermis with a score >0 was grouped as pemphigus with eosinophilic infiltrate (E+). Those without any eosinophilic infiltrate (score = 0 for pustules/crust, follicles, and dermis) were grouped as pemphigus without eosinophilic infiltrate (E–).

Disease outcome was classified as no improvement, improvement or remission. The final disease outcome was dichotomized as no improvement or improvement (including improvement or remission). Survival was dichotomized as survival or death (including dogs that were euthanized).

For exploration of histological features, eosinophilic infiltrates in either the pustules/crust, follicles or dermis with a score >0 were classed as eosinophilic. Neutrophilic infiltrates in either the pustules/crust, follicles or dermis with a score >0 were classed as neutrophilic. Any active acantholysis in the pustules/crusts or follicles >0 was described as active acantholysis, and any acantholytic lesion in the pustules/crusts or follicles >0 was described as acantholytic.

Associations between group (E+ versus E–) and categorical variables were tested using Fisher's exact test with a two-sided hypothesis of no association rejected at $P \leq 0.05$. Where the table was 2 × 2, the odds ratio (OR) was used to describe the association with a 95% confidence interval excluding 1.0. When a single proportion was evaluated, a test of binomial proportions was performed. Numeric responses were compared across groups using Mann–Whitney U-tests with a two-sided null hypothesis rejected at $P \leq 0.05$.

Multivariate analysis was performed to explore any associations of disease features with the presence of eosinophilic infiltrate (E+ versus E–) using logistic regression with a stepwise entry at $P < 0.3$ and variables kept in the model at $P \leq 0.05$. Significant variables associated with the response were described using the OR with a 95% confidence interval excluding 1.0. Similar analysis was performed on the response of final disease outcome and on survival. All analyses were performed using SAS statistical software (version 9.1; SAS institute, Cary, NC, USA).

Results

History and signalment

Forty dogs with PF evaluated between 2002 and 2008 were included in the study (Table 1). Thirty-one were pure-bred dogs and nine were mixed-breed. English bulldogs were the most common breed ($n = 5$), followed by Boston terriers ($n = 3$). Dachshunds, cocker spaniels, Yorkshire terriers and Labrador retrievers were represented by two dogs each. The remaining 15 breeds were represented by single dogs. Twenty-three dogs (57.5%) were male (7 intact) and 17 dogs (42.5%) were female (5 intact). Twelve of the 17 female dogs (71%) and 13 of 23 males (57%) were E+. There was no statistically significant difference in the frequency of males versus females for dogs with or without an eosinophilic infiltrate ($P = 0.51$).

The median age at the time of diagnosis for E+ dogs was 7 years, with a range of 0.25–13 years and a mean of 7.3 years. For E– dogs, the median age at the time of diagnosis was 7 years, with a range of 1–12 years and a mean of 6.4 years. The duration of clinical signs for E+ dogs prior to diagnosis ranged from 1 to 52 weeks, with a median of 8 weeks and a mean of 14.8 weeks. For E– dogs, the duration of clinical signs prior to diagnosis ranged from 1 to 52 weeks, with a median of 14 weeks and a mean of 15.4 weeks. The duration of clinical signs for one E– dog (case 22) was not available. There was no statistically significant difference in the age of dogs ($P = 0.47$) or the time to diagnosis ($P = 0.77$) in dogs with or without an eosinophilic infiltrate. Pemphigus foliaceus was diagnosed most commonly in the summer (16 dogs, 40%), followed by autumn (11 dogs, 27.5%), spring (ten dogs, 25%) and winter (three dogs, 7.5%). There was no statistically significant difference in the frequency of diagnosis across seasons for dogs with or without eosinophilic infiltrate ($P = 0.18$).

Fifteen of 40 dogs (37.5%) had a history consistent with allergic dermatitis. These included: flea allergy in

Table 1. Clinical features, treatment and outcome of dogs with pemphigus foliaceus

Case	Breed	Age (years)	Sex	Allergic disease	TDx* (weeks)	Initial treatment	Maintenance treatment	Outcome	E+ versus E−	TRem (weeks)	TFoll (weeks)
1	Miniature Schnauzer	11	MN	No	4	AB GC	AB GC	NI/D	E−	NA	2
2	West Highland white terrier	7	F	Yes	20	AB GC AZ	AB GC AZ	R	E−	6	208
3	Boston terrier	3	M	No	1	AB GC	AB GC	R	E+	8	16
4	Rat terrier	3	FS	No	2	AB GC AZ	AB GC AZ	I	E+	NA	20
5	Akita	5	MN	Yes	24	AB	NA	NA	E+	NA	NA
6	Bulldog	1	MN	No	12	AB GC AZ	AB GC AZ	R	E−	6	20
7	Akita	6	FS	No	52	AB GC AZ	AB GC AZ	I	E+	NA	104
8	Labrador retriever	5	FS	Yes	8	AB GC AZ	AB GC AZ	R	E+	4	104
9	Miniature pinscher	6	FS	Yes	4	AB GC	AB GC	R	E+	4	20
10	English bulldog	2	M	Yes	16	AB GC AZ	GS	R/D	E−	4	28
11	Mixed breed	7	M	No	2	AB GC AZ	AB GC AZ	NI/D	E+	NA	4
12	Hound	0.25	M	No	6	AB GC AZ	GS	R	E+	24	104
13	Dachshund	4	MN	No	3	AB GC AZ	AB GC AZ	R	E−	6	12
14	Chihuahua	7	FS	No	2	AB GC AZ	AB GC CsA	NI/D	E+	NA	12
15	Mixed breed	9	FS	No	40	AB GC AZ	GS TN	R	E+	10	78
16	Schipperke	9	FS	Yes	8	AB GC AZ	AB GC AZ	R	E+	12	78
17	Mixed breed	8	MN	No	2	AB GC	AB GC	R	E+	6	6
18	Standard poodle	13	MN	No	6	AB GC	AB GC	NA	E+	NA	NA
19	English bulldog	3	F	Yes	12	AB GC	AB GC	R	E+	8	12
20	Labrador retriever	6	MN	No	4	AB GC	AB GC	I	E+	NA	6
21	Mixed breed	8	MN	No	24	AB GC	AB GC	R	E+	4	12
22	Lhasa Apso	9	MN	No	NA	AB GC	AB GC CH	R	E−	12	12
23	Shih Tzu	11	FS	Yes	28	AB GC	AB GC AZ	R	E+	12	52
24	Boston terrier	12	FS	No	32	AB	AB	D	E+	NA	1
25	Doberman pinscher	12	MN	No	12	AB GC	AB GC	R	E+	4	16
26	Mixed breed	12	MN	No	12	AB GC	AB GC AZ	R/D	E−	4	20
27	Cocker spaniel	8	FS	No	52	AB GC	AB GC	I	E−	NA	4
28	Mixed breed	4	MN	Yes	24	AB GC	AB GC	R	E+	4	4
29	Pekeapoo	11	M	No	4	AB	AB	R	E−	4	208
30	English bulldog	3	MN	No	24	AB GC	NA	NA	E−	NA	NA
31	Dachshund	4	M	No	16	AB GC	AB GC	R	E−	6	32
32	Mixed breed	10	M	No	12	AB GC	AB GC AZ	I/D	E+	NA	24
33	Yorkshire terrier	3	FS	Yes	1	AB GC	AB GC	R	E−	4	104
34	English bulldog	8	FS	Yes	20	AB GC	AB GC	R	E−	6	36
35	Cocker spaniel	5	FS	No	8	AB GC	AB GC	R	E−	6	12
36	Yorkshire terrier	7	MN	Yes	16	AB GC	AB GC	R	E+	6	12
37	Mixed breed	13	MN	Yes	28	AB GC	AB GC	R	E+	6	78
38	Boxer	10	MN	No	28	AB GC AZ	GS PX	R	E+	4	78
39	Mixed breed	3	FS	Yes	8	AB GC AZ	AB GC AZ	R	E+	12	40
40	Boston terrier	10	FS	Yes	8	AB GC AZ	AB GC AZ	R	E+	6	12

*TDx, duration in weeks from lesion onset to diagnosis; E+, eosinophilic infiltrate; E−, noneosinophilic infiltrate; TRem, time to remission; M, male; MN, neutered male; F, female; FS, spayed female; AB, antibiotic; AZ, azathioprine; CH, chlorambucil; CsA, ciclosporin; GC, glucocorticoid; GS, gold salts; PX, pentoxifylline; TN, tetracycline and niacinamide; I, improvement; I/D, improvement and died/euthanized; NA, not available or not applicable; NI, no improvement; NI/D, no improvement and died/euthanized; R, remission; R/D, remission and died/euthanized.

nine dogs (60%), seasonally recurrent pyoderma in three dogs (20%), pododermatitis in three dogs (20%), recurrent otitis externa in two dogs (13.33%) and angioedema in one dog (6.67%). Of the 15 dogs with allergic dermatitis a significant proportion, 11 of 15 (73%), were E+ ($P = 0.04$). Two dogs had a history of previous dermatological problems not related to allergic disease. One of these dogs had a history of juvenile-onset demodicosis, alopecia areata and sterile granuloma pyogranuloma syndrome (case 13). The other dog presented with cyclic flank alopecia (case 38). Drug-induced PF was suspected in one dog, which was classified as E+. Eight of 40 dogs (20%) had a concurrent systemic disease process at the time of diagnosis, with acute renal failure, intervertebral disc disease, brachycephalic airway syndrome, hypoadrenocorticism, mitral insufficiency and a urinary tract infection affecting one dog each. Two dogs had a history of idiopathic epilepsy. Of the eight total dogs with a concurrent disease a significant proportion, five of the eight (63%), were E+ ($P = 0.01$).

Of the 25 E+ dogs, antihistamines, glucocorticoids and antibiotics were administered to 10 (40%), 16 (64%) and 20 dogs (80%) respectively. Of the 15 E− dogs, antihistamines, glucocorticoids and antibiotics were administered to three (20%), eight (53%) and 12 dogs (80%) respectively. There was no statistically significant difference in administration of antihistamines ($P = 0.53$), glucocorticoids ($P = 0.30$) or antibiotics ($P = 1.0$) prior to the diagnosis of PF for dogs with or without an eosinophilic infiltrate. Nineteen of 40 dogs (47.5%) received other drugs, including systemic antifungal medications in eight dogs, levothyroxine in five dogs and ivermectin in two dogs. One dog received each of the following: phenobarbital, a nonsteroidal anti-inflammatory drug, tetracycline and niacinamide and potassium bromide.

Clinical findings

Thirty-three dogs (82.5%) presented with lesions affecting the face (including the dorsal muzzle, head, periocular and/or preauricular regions), 34 dogs (85%) with pinnal lesions, 36 dogs (90%) with lesions affecting the dorsum and 24 dogs (60%) with lesions affecting the ventrum. Lesions on the feet (dorsal aspect of paw and interdigital regions) and limbs were seen in 22 (55%) and 11 (27.5%) dogs respectively. There was no statistically significant difference in the frequency of lesions on the face ($P = 1.0$), pinnae ($P = 0.38$), dorsum ($P = 1.0$), ventrum ($P = 0.38$), feet ($P = 0.75$) or limbs ($P = 0.71$) for dogs with or without eosinophilic infiltrate. Pustules were described in 31 dogs (77.5%), crusting dermatitis in 40 dogs (100%), epidermal collarettes in 38 dogs (95%), alopecia in 29 dogs (72.5%) and papules in 16 dogs (40%). There was no statistically significant difference in the frequency of the pustules ($P = 0.44$), collarettes ($P = 0.44$), papules ($P = 0.74$) and alopecia ($P = 0.72$) for dogs with or without eosinophilic infiltrate. The frequency of crusts could not be compared since all dogs had crusts. Other dermatological lesions included footpad hyperkeratosis in 11 dogs (27.5%), generalized seborrhoea in two dogs (5%), and nasal planum depigmentation and loss of architecture of the nasal planum in one dog each (2.5%). Pruritus was noted in 32 dogs (80%), lethargy in ten dogs

(25%), anorexia in three dogs (7.5%) and fever in eight dogs (20%). There was no statistically significant difference in the frequency of pruritus ($P = 1.0$), lethargy ($P = 0.71$), anorexia ($P = 0.54$) or fever ($P = 0.68$) for dogs with or without eosinophilic infiltrate.

A complete blood count and biochemical profile were performed in 28 dogs prior to initiation of therapy. Of these dogs, 18 dogs exhibited neutrophilic leukocytosis and seven exhibited eosinophilia. Five of the seven dogs with eosinophilia and 11 of the 18 dogs with neutrophilic leukocytosis were E+. There was no statistically significant difference in the frequency of peripheral eosinophilia ($P = 0.69$) or neutrophilia ($P = 1.0$) for dogs with or without an eosinophilic infiltrate. Of the 28 dogs, abnormalities on serum biochemical analysis revealed hypoalbuminemia in seven dogs, increased serum alkaline phosphatase (ALP) in six dogs, increased serum alanine transaminase (ALT) in one dog, hypercholesterolemia in one dog and azotemia in one dog.

Treatment and outcome

Antibiotics were administered to all 40 dogs and were the only therapy given to three dogs (7.5%). The most commonly prescribed initial treatment for PF was glucocorticoid monotherapy in 22 dogs (55%). Fifteen dogs (37.5%) received a single glucocorticoid and azathioprine. Eleven of the 37 dogs treated with immunosuppressive drugs received adjunctive medications including famotidine in six dogs (55%), pentoxifylline in two dogs (18.2%), ivermectin in two dogs (18.2%) and tramadol in one dog (9.1%). Adverse reactions to initial medications were recorded in 17 (46%) of the 37 dogs treated with immunosuppressive drugs. Adverse effects included clinical signs of iatrogenic hyperadrenocorticism (polyuria, polydipsia, cutaneous atrophy, muscle wasting etc.) in ten dogs, hepatotoxicity (increased ALT and/or ALP) in five dogs, anaemia and thrombocytopaenia in two dogs and a urinary tract infection in two dogs. Leucopenia, vomiting, calcinosis cutis and diabetes mellitus occurred in one dog each. Of the dogs that experienced adverse effects following immunosuppressive therapy, a significant proportion, 14 of 17 (82%), were E+ ($P = 0.05$).

Two of the three dogs prescribed an antibiotic only did not return for re-evaluation following diagnosis. The other dog died prior to re-evaluation. Of the 37 dogs prescribed immunosuppressive therapy, remission occurred in 27 dogs (73%), improvement in four dogs (11%) and no improvement in three dogs (8%). The outcome for three dogs was not available. There was no statistically significant difference in disease outcome (remission, improvement or no improvement) for dogs with or without eosinophilic infiltrate ($P = 0.53$). The median time to remission for E+ dogs was 6 weeks, with a range of 4–24 weeks and a mean of 8 weeks. For E− dogs, the median time to remission was 6 weeks, with a range of 4–12 weeks and a mean of 6 weeks There was no statistically significant difference in the time to remission for dogs with or without eosinophilic infiltrate ($P = 0.17$). Seven of 40 dogs (17.5%) later died or were euthanized. Four of the seven (57%) were E+ dogs, but there was no statistically significant difference in the frequency of survival in dogs with or without eosinophilic infiltrate

(P = 0.62). The median follow-up time for all dogs was 20 weeks, with a range of 1–208 weeks and a mean of 43 weeks.

Histopathology

A total of 25 of 40 dogs (63%) had an eosinophilic infiltrate score >0 (i.e. E+) in either the pustules/crust, follicles or dermis. Of these 25 dogs, eosinophils in the pustules/crust were scored as 0 in three dogs, 1+ in five dogs, 2+ in six dogs, 3+ in seven dogs and 4+ in two dogs. Twenty-four dogs had follicular infundibular pustules. Of these dogs, eosinophils in the follicular infundibulum were scored as 0 in eight dogs, 1+ in five dogs, 2+ in four dogs, 3+ in five dogs and 4+ in two dogs. Eosinophil scores were >0 in the superficial dermis of all 25 dogs, and were scored as 1+ in seven dogs, 2+ in six dogs, 3+ in 11 dogs and 4+ in one dog. Eighteen of the 25 dogs (72%) had a greater than 40% eosinophilic component to their disease (scored 2+ or greater) in the pustules/crust, follicles or dermis. In 16 of 25 dogs, estimates of the number of eosinophils were greater than or equal to neutrophils in the pustules/crust, follicles or dermis.

Neutrophils were reported in the pustules/crust, follicles or dermis of all 40 dogs, preventing analysis of the frequency of any neutrophilic infiltrate. Neutrophils in the pustules/crust were scored as 0 in three dogs, 1+ in four dogs, 2+ in eight dogs, 3+ in six dogs and 4+ in 17 dogs. In two dogs, the crust was too degenerate to identify inflammatory cells. Twenty-four dogs had follicular infundibular pustules. Of these dogs, neutrophils were scored as 0 in three dogs, 1+ in five dogs, 2+ in three dogs, 3+ in seven dogs and 4+ in six dogs. Neutrophils were scored >0 in the dermis of all 40 dogs. A neutrophil score of 1, 2 or 3 in either the pustules/crust, follicles or dermis occurred more frequently in E+ dogs. In contrast, neutrophil scores of 4 were more frequent in E– dogs (P = 0.001).

Acantholytic cells were seen in all 40 dogs in either the pustules/crust or follicular infundibulum. Active acantholysis in either the pustules/crust or follicular infundibulum was seen in 31 of 40 dogs (78%). Eighteen of 25 E+ dogs (72%), and 13 of 15 E– dogs (87%) exhibited active acantholysis. There was no statistically significant difference in the frequency of active acantholysis (P = 0.20) or acantholytic cells (P = 0.33) for dogs with or without an eosinophilic infiltrate.

Effect on survival

Signalment, history, histological features and disease outcome (improvement, remission, no improvement) were not associated with survival for dogs with or without an eosinophilic infiltrate. Exploration of any association of signalment, history, and histological features, including the presence of eosinophilic infiltrate (E+), with survival in all dogs revealed that anorexic dogs were significantly more likely to die or be euthanized (OR = 30.0; 95% CI 1.8–490.7).

Discussion

The clinical importance of specific tissue cell populations has been investigated as a diagnostic tool, mechanism of pathogenesis and prognostic indicator in humans.[12–25] These studies demonstrate the importance of classifying and quantifying cellular tissue infiltrates. However, to the authors' knowledge, this is the first study to evaluate the cellular infiltrate at different microanatomic locations in canine PF, and to compare different cellular infiltrates with clinical features and outcomes.

Eosinophils can be present in variable numbers in canine PF.[5,10] In the current investigation, an eosinophilic infiltrate, although not a consistent feature, was present in 25 of 40 dogs (63%) with PF. The identity of the major antigen(s) in canine PF is unknown, although genetic factors play a role in disease developement.[5,26] In humans, the pathogenesis of autoimmune blistering diseases is multifactorial.[20] Cell-mediated immune responses, in addition to autoantibodies, contribute to lesion formation.[20] The type of cellular infiltrate in these diseases is dependent upon specific chemokines, chemokine receptors and adhesion molecules.[20] To illustrate the importance of the cellular infiltrate, autoantibodies alone are not sufficient for disease manifestation in people with pemphigus gestationis, which requires eosinophils to induce blister formation.[21] Therefore, factors affecting eosinophil homing and activation influence lesion appearance in this condition. Eosinophils are recruited to tissues by T helper 2 (Th2)-type cytokines, including interleukins (IL)-4, 5, 10, and 13.[20,21] Chemotactic factors, including eotaxin and RANTES (Regulated on Activation Normal T-cell Expressed and Secreted) also have a role in eosinophil infiltration.[12,21,27,28] Once in the tissue, eosinophils release granule proteins and metalloproteinases, contributing to tissue destruction and blister formation.[21] To the authors' knowledge, this is the first study to determine whether eosinophils, or another type of inflammatory cell, affect disease manifestation in dogs with an autoimmune disease.

A higher incidence of PF in dogs with allergic skin disease has been cited in the literature.[1] Equine PF has been reported in a group of horses with *Culicoides* hypersensitivity,[6,29] and several cats with PF in one study also had presumed allergic dermatitis.[3] In humans, exposure to black fly bites has been associated with an endemic form of PF.[30] Interestingly, a statistically significant proportion of the dogs in our study with allergic skin disease had an eosinophilic infiltrate as compared to dogs without allergic skin disease. Nine of the 15 dogs with allergic skin disease had a history of flea allergy dermatitis. Flea allergy has previously been reported as the most common skin disease present in dogs later diagnosed with PF.[31] In one dog in our study (case 16; E+), flea allergy immediately preceded the outbreak of PF. This dog was treated with fipronil (Frontline® Spray; Merial) for 3 months prior to the onset of PF for severe flea allergy dermatitis based on classic clinical signs (i.e. caudodorsal dermatitis and pruritus) and the presence of fleas. Facial angioedema occurred in one dog (case 40; E+) approximately 6 weeks prior to development of PF lesions and 10 days following vaccination for rabies, distemper, infectious hepatitis, parvovirus and leptospirosis. Angioedema can be caused by numerous factors, but is nearly always associated with hypersensitivity reactions.[32] However, an association of disease manifestation with vaccination cannot be conclu-

sively made. Only one dog in our study (case 3; E+) had features suggestive of drug-induced PF. This dog developed pustular dermatitis 1 week following surgery for removal of a cervical thoracic mass. Suspect drugs in this dog included ampicillin, amoxicillin, enrofloxacin and metronidazole. Despite withdrawal and future avoidance of these drugs, glucocorticoid treatment was necessary in order to induce remission. Autoantibodies may result from an altered or abnormal immune regulation or antigenic stimulation.[8] As such, any factor (i.e. drugs, allergens, vaccination) with potential immunomodulatory effects could promote autoantibody production. Further investigations are warranted to investigate allergic disease as a causative factor of PF in domestic animals.

Environmental factors such as UV light exposure may induce flares of PF in dogs.[5] Although a seasonal pattern of PF was not found in one study of 66 dogs with PF,[31] lesional scores of PF worsened in the summer and improved in the winter in another investigation.[33] In horses, a higher incidence of PF flares during fall (autumn) and winter was reported by Vandenabelle *et al.*,[6] but in another study, a clear seasonal association was not reported.[34] Since seasonality may also correlate with allergic flares, this parameter was evaluated in dogs in our study. The majority of dogs (27/40 dogs; 68%) were diagnosed in either the spring or summer. However, clear associations of seasonality are difficult to assess due to the subtropical climate and overlap of seasons in our geographic region.

Bacterial pyoderma can be a concurrent finding in dogs with PF,[1,4] and dogs with pyoderma were not excluded from this study. However, the clinical and histopathological criteria used in this investigation[5] were diagnostic of PF in all dogs. Concomitant endoparasitism was not evaluated in this study since faecal examination and heartworm testing are not part of a typical PF work-up. Although endoparasites can certainly cause eosinophilia, the retrospective nature of this investigation precluded these diagnostics. Dogs with an eosinophilic infiltrate, however, were more likely to have a concurrent systemic disease. Of the eight total dogs with a concurrent systemic disease, five were E+ (63%). Medications utilized to treat underlying disease prior to the diagnosis of PF in these dogs included potassium bromide, itraconazole, levothyroxine, phenobarbital and furosemide. None of these medications have been directly associated with eosinophilia. Adverse reactions to various drugs could result in a superficial pustular drug reaction with prominent eosinophils.[10] However, none of the E+ dogs on medication for an underlying systemic disease had a clinical or histopathological appearance compatible with a drug reaction.

Adverse effects from immunosuppressive drugs were more likely to occur in dogs with an eosinophilic infiltrate. Of the 17 dogs for which an adverse effect was recorded, 14 were E+ dogs (82%). Although this is an interesting finding, the adverse effects noted in this study may have been caused as a direct result of the drugs themselves and the doses necessary to achieve therapeutic effect.

Exploration of any variable with survival revealed the odds of death were 30 times higher in dogs with anorexia. However, due to the wide confidence interval

(95%, 1.8–490.7), the overall reliability of this estimate is questionable. One possible explanation may be that sicker dogs are more likely to be anorexic, thus are more likely to die or be euthanized.

One limitation of the study design was that dogs receiving medications prior to diagnosis of PF were not excluded from the investigation. Unfortunately, controlling all variables that impact data cannot be accomplished in a retrospective study. The design of this study was similar to other retrospective PF studies,[3,4,7] in that medications given prior to diagnosis were recorded, but these animals were not excluded. In cats, administration of glucocorticoids prior to or at the time of biopsy affected the percentage of diagnostic biopsy specimens in one investigation.[3] In our study, glucocorticoids had been given to 24 of 40 dogs (16 E+ cases) prior to diagnosis, and antihistamines had been administered to 13 of 40 dogs (10 E+ cases). Glucocorticoids are potent immunosuppressive drugs, possess anti-inflammatory properties and are also the most effective drug at inhibiting eosinophilic inflammation.[35,36] Antihistamines can also have an anti-eosinophilic effect in human.[36] It is interesting to note that despite treatments that can interfere with the histopathological diagnosis of PF and alter the presence of inflammatory cells, numerous dogs in this study – including those with an eosinophilic infiltrate – received such therapy.

In our study, there was no statistically significant difference in the occurrence of acantholytic cells or active acantholysis in dogs with or without an eosinophilic infiltrate. However, no description of the type, number or location of inflammatory cells present in PF lesions has been described for dogs with both allergic disease and PF. In one study of cats, there was no difference in the cellular type or intensity of dermal infiltrate in PF lesions between animals with or without a history of allergic dermatitis.[3] Eosinophils often predominate within dermal and perifollicular infiltrates of dogs with allergic dermatitis[38] and may also be seen in intraepidermal pustules.[37,38] In our study, of the 15 dogs with allergic dermatitis, 11 (73%) were E+ and contained an eosinophilic infiltrate in either the pustules/crust, follicles, or dermis.

One dog in this investigation (case 24) had an eosinophilic infiltrate and spongiosis on histopathological evaluation, but no active acantholysis and very few acantholytic cells. Prominent eosinophilic spongiosis without acantholysis has been reported to be an uncommon presentation of canine PF.[10] It is thought that this variant represents an early form of PF, with more typical histological features appearing in later stages.[10] In this particular dog, however, clinical lesions had been present for 32 weeks prior to diagnosis. Similarly, an additional dog (case 23) also had very few acantholytic cells, no active acantholysis, and prominent eosinophilic spongiosis and eosinophilic infiltration at all microanatomic levels. Similar to case 24, this dog exhibited clinical PF lesions for an extended duration (28 weeks) prior to diagnosis. Neither dog received glucocorticoids prior to biopsy. Although only two dogs in this retrospective study exhibited an eosinophilic, non-acantholytic form of PF, both dogs had lesions for several months prior to diagnosis. It is possible, however, that since pustules can manifest in 'waves'[10] the lesions biop-

sied in these dogs represented early individual lesions of an entire disease process. Although eosinophilic spongiosis in people can be the hallmark of early autoimmune disease,[18] further studies are warranted to see whether this is true in dogs.

In conclusion, there were no statistically significant differences noted in signalment, clinical distribution or dermatological lesions, previous medications, haematological values, time to diagnosis, season of diagnosis, the presence of acantholytic cells or acantholysis, treatment, response to treatment or final disease outcome in dogs with PF with or without an eosinophilic infiltrate. However, our findings suggest there may be a relationship between eosinophilic infiltrate and allergic skin disease, systemic disease and adverse effects of immunosuppressive therapy. Further studies are warranted to evaluate chemotactic cytokines in pustules and sera of affected animals, as knowledge of specific cytokine expression in PF may further understanding of its pathogenesis, and be used to monitor disease activity, lessen disease severity and provide new targets for therapy.

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Résumé L'importance de l'infiltrat cellulaire tissulaire a été étudiée comme outil diagnostique, mécanisme de pathogénie et indicateur pronostic dans les processus pathologiques de l'homme. Les éosinophiles, en particulier, ont un rôle précis dans les manifestations des lésions cutanées des dermatites auto-immunes de l'homme. L'identification d'un infiltrat éosinophilique peut aider au diagnostic d'une maladie bulleuse dans ses premiers stades. Chez les chiens atteins de pemphigus foliacé, les éosinophiles peuvent être présents à des degrés variables dans les tissus lésionnels. Cette étude évalue rétrospectivement 40 chiens atteints de pemphigus foliacé et examine les données cliniques et histopathologiques, et le pronostic dans les cas avec ou sans infiltrat éosinophilique. Vingt-cinq chiens sur 40 (63%) montraient un infiltrat éosinophilique au niveau de pustules/croutes, de l'infundibulum folliculaire ou du derme. Il n'y avait pas de différence statistique significative dans la nature ou la distribution des lésions dermatologiques, la réponse au traitement ou la résolution de la maladie avec ou sans infiltrat éosinophilique. Cependant, les chiens atteints d'une maladie concomitante présentaient significativement plus souvent un infiltrat éosinophilique ($P = 0.01$). De même, les chiens ayant montré des effets secondaires à la thérapeutique immunosuppressive avaient plus fréquemment un infiltrat éosinophilique ($P = 0.05$). Quinze chiens sur 40 (38%) avaient un antécédent de maladie allergique et une proportion significativement plus élevée de ces animaux avait un infiltrat éosinophilique ($P = 0.04$). D'autres études sont nécessaires pour établir le rôle des éosinophiles dans la pathogénie, les stratégies thérapeutiques et le pronostic du pemphigus foliacé chez le chien.

Resumen La importancia de los infiltrados celulares en tejido se ha investigado como un instrumento de diagnóstico, mecanismo de patogénesis, e indicador pronóstico en enfermedades humanas. Los eosinófilos en particular, tienen un papel diferenciado en las manifestaciones de lesiones cutáneas en enfermedades autoinmunes en personas. La identificación de un infiltrado eosinofílico puede ayudar en el diagnóstico de enfermedades vesiculares inmunomediadas en fases tempranas de la enfermedad. En perros con pénfigo foliáceo, los eosinófilos pueden estar presentes en diverso grado en el tejido lesionado. Este estudio retrospectivo evaluó 40 perros con pénfigo foliáceo y examinó las características clínicas e histopatológicas, así como el resultado final en casos con y sin infiltrado eosinofílico. Veinticinco de los cuarenta perros (63%) tenían infiltrado de eosinófilos bien en las pústulas/costras, en el infundibulo folicular o en la dermis. No hubo diferencia estadísticamente significativa en la distribución clínica ni en el aspecto de las lesiones de la piel, respuesta al tratamiento o resultado de la enfermedad en perros con o sin infiltrado de eosinófilos. Sin embardo perros con una enfermedad asociada tenian una mayor probabilidad significativa de presentar un infiltrado eosinofílico ($P = 0,01$). De la misma forma, perros con efectos adversos asociados con el tratamiento inmunosupresor tenían mayor probabilidad de presentar un infiltrado eosinofílico ($P = 0,05$). Quince de los cuarenta perros (38%) tenían una historia de enfermedades alérgicas y una proporción significativa de estos perros tenían un infiltrado de eosinófilos ($P = 0,04$). Hacen falta más estudios para investigar el papel de los eosinófilos en la patogenia de la enfermedad, estrategias terapéuticas y pronóstico en perros con pénfigo foliaceo.

Zusammenfassung Die Bedeutung von zellulären Infiltraten im Gewebe hat man als diagnostische Hilfsmittel, als Mechanismen der Pathogenese und als prognostischer Indikator bei Krankheitsprozessen der Menschen bereits untersucht. Vor allem Eosinophile spielen eine bedeutende Rolle bei der Manifestation von Hautläsionen bei Autoimmunerkrankungen des Menschen. Das Erkennen eines eosinophilen Infiltrates kann bei der Diagnose von bullösen Autoimmunerkrankungen bei frühen Veränderungen im Krankheitsverlauf hilfreich sein. Bei Hunden mit Pemphigus foliaceus können Eosinophile in unterschiedlichem Ausmaß im veränderten Gewebe vorkommen. Diese retrospektive Studie evaluierte 40 Hunde mit Pemphigus foliaceus und untersuchte klinische und histopathologische Charakteristika und den letztendlichen Ausgang der Erkrankung in Fällen mit und ohne eosinophilen Infiltraten. Fünfundzwanzig von 40 Hunden (63%) hatten ein eosinophiles Infiltrat entweder in den Pusteln/Krusten, im follikulären Infundibulum oder in der Dermis. Es bestand kein signifikanter Unterschied in der klinischen Verteilung oder dem Aussehen der dermatologischen Veränderungen, im Ansprechen auf die Behandlung oder beim Ausgang

der Erkrankung bei Hunden mit oder ohne eosinophilem Infiltrat. Nichtsdestotrotz wiesen Hunde mit einer zusätzlichen Erkrankung mit höherer Wahrscheinlichkeit ein eosinophiles Infiltrat auf ($P = 0,01$). In ähnlicher Form hatten Hunde, die Nebenwirkungen auf immunsuppressive Therapie zeigten, mit höherer Wahrscheinlichkeit ein eosinophiles Infiltrat ($P = 0,05$). Fünfzehn von 40 Hunden (38%) hatten einen Vorbericht von allergischer Erkrankung und ein signifikant höherer Anteil dieser Hunde zeigte ein eosinophiles Infiltrat ($P = 0,04$). Weitere Studien sind nötig, um die Rolle der Eosinophilen in der Pathogenese von Krankheiten, für therapeutische Strategien und für die Prognose von Hunden mit Pemphigus foliaceus zu untersuchen.