Prospective Study of Familial Canine Dermatomyositis

Correlation of the Severity of Dermatomyositis and Circulating Immune Complex Levels

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Familial canine dermatomyositis in collie dogs is a newly recognized spontaneous disease that resembles dermatomyositis in children. A litter of 9 collie dogs was studied from birth to 7.5 months of age. The onset and severity of dermatitis and myositis correlated with elevated serum levels of circulating immune complexes (CICs) and IgG. The immunoglobulin component of the CICs consisted principally of IgG. All dogs developed elevated levels of CIC before or concurrent with the onset of dermatitis. Myositis developed later. The CIC tended to peak between 14–18 weeks of age in all dogs except the most severely affected dog, in which the CICs continued to increase to 238 μg/ml (controls 30 μg/ml) at 7 months of age. In the moderately affected dogs the CICs tended to stabilize at the levels reached at 14–18 weeks, and in the mildly affected dogs the CICs tended to decrease to normal levels after 14–18 weeks. Although the dogs had electromyographic and repetitive nerve stimulation abnormalities, the abnormalities did not correlate with severity of dermatomyositis or degree of elevation of CICs or IgG. Necropsy at 7.5 months of age revealed that all dogs had myositis and 8 of 9 had dermatitis. Except for 1 dog, the severity of dermatomyositis correlated positively with higher levels of CICs. A strong positive association between elevated levels of CICs and IgG, but not IgM or IgA, was generally present. Elevated levels of CICs appear to be involved in the mechanisms that control the development, severity, and progression of dermatomyositis in collie dogs. (Am J Pathol 1986, 123:465–479)

DERMATOMYOSITIS is an inflammatory muscular, cutaneous, and sometimes vascular human connective tissue disease.[1-5] Neither its cause nor its pathogenesis is known.[5] Although viruses and a few other infectious agents have been implicated in the causation of dermatomyositis,[6-10] infectious agents generally are not isolated from tissues of affected patients.[5] Dermatomyositis may be one of the autoimmune diseases[11] involving both cell-mediated[12] and humoral immunity.[13] There is also a suggested genetic basis for dermatomyositis.[11,14,15] Human dermatomyositis develops in both adults and children.

We recently described a familial disease in juvenile collie dogs characterized by dermatitis and myositis that resembles dermatomyositis in children.[16-18] The detailed postmortem findings from the dogs in this study, as well as from other collie and collie-crossbred dogs, are presented in an accompanying article.[19] The purpose of this study was to evaluate the relationship among levels of circulating immune complexes, serum immunoglobulin levels, and the severity of skin and muscle lesions.

Materials and Methods

A litter of 12 collie pups, produced by the breeding of 2 purebred collie dogs affected with dermatomyositis, was studied. The sire and dam of this litter were described in previous publications[17,18] in which the par-

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ents were identified as A and B, respectively. Three pups died within 5 days of birth, and the postmortem findings are presented elsewhere.[19] The remaining 9 dogs, 4 females and 5 males, were assigned number 31 through 39, and were evaluated from birth to 7.5 months of age. All dogs were vaccinated for canine distemper, adenovirus, and leptospirosis at 7.5, 13, and 16 weeks of age. Vaccine against parvovirus was administered at 13 and 16 weeks of age. All dogs were given pyrantel pamoate to eliminate intestinal parasites at 6 and 12 weeks of age. Dermatologic examinations were performed twice a week from 20 days to 3 months of age and once a week from 3 to 7.5 months of age. General physical examinations were performed monthly. Blood was collected at 2-week intervals, and serum was frozen at −70 C.

Electromyograms (EMGs) and biopsy samples of temporalis and extensor carpi radialis muscles were performed at 6 weeks of age. The EMGs were performed on the left side and muscle biopsies on the right side. The EMGs were performed as previously described.[17] Anesthesia was induced with intravenous thiamylal (Bio-Ceutic Laboratories, Inc., Fort Dodge, Iowa), and was maintained with halothane (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa). One male and 2 female German shepherd pups 11 weeks of age were used as controls for muscle biopsy and electromyography. One female and 3 male weimaraner–Doberman pinscher crossbred pups 7 weeks of age were used as controls for electromyography. Skin and muscle biopsies and EMGs were performed again on each collie between 9 and 12 weeks of age. The EMGs were performed on the left side, and skin was collected from areas of dermatitis. Right temporalis and extensor carpi radialis muscles were collected. Two additional skin specimens were collected from areas of erythema, crusting or scaling on the ears from each dog between 9 and 12 weeks of age. The specimens were immediately placed in Michel's medium for immunofluorescence evaluation for IgG and complement component 3 (C3). The cutaneous samples were washed in Michel's buffer and frozen in isopentane chilled in liquid nitrogen, and stored at −70 C. Frozen skin specimens were mounted in cryostat gel (Lab-Tek Products, Naperville, Ill), and 4-μ serial sections were mounted on glass slides, fixed in acetone, and stained with fluorescein isothiocyanate (FITC)-conjugated sheep anti-canine IgG.[20] Identically prepared frozen sections were incubated for 30 minutes with sheep anti-canine C3,[21] washed, and stained with FITC-rabbit anti-sheep IgG (Miles-Yeda, Rehovot, Israel).

A third set of EMGs and muscle biopsies were performed on each dog between 20 and 23 weeks of age. The EMGs were performed on the right side, and left temporalis and extensor carpi radialis muscles were biopsied. The skin and muscle specimens collected at 6 weeks, 9–12 weeks, and 20–23 weeks of age were placed in 10% neutral buffered formalin (NBF) and processed in an automatic tissue processor. Tissues were sectioned at 6 μ, stained with hematoxylin and eosin (H&E), and evaluated histopathologically.

A fourth set of EMGs was performed just before the dogs reached 7.5 months of age. In addition, repetitive nerve stimulation (RNS) studies were performed at this time. Electrical stimuli were applied in a rectangular pulse lasting 0.1 ms at 2, 5, and 10 Hz. At least 15 seconds were allowed between each RNS. A minimum of 5 evoked compound muscle action potentials were recorded following RNS. Near-nerve temperature was recorded immediately after nerve stimulation studies with a transcutaneous probe (Electromedics Inc, Englewood, Co).

At 7.5 months of age each dog was euthanatized with an intravenous overdose of sodium pentobarbital, and a postmortem examination was performed. Detailed postmorten findings in organs other than skin and muscle are presented elsewhere.[19] Portions of skin from face and carpus, and portions of the longissimus, hyopharyngeus or thyropharyngeus, intercostal, temporalis, masseter, extensor digitorum longus, biceps femoris, diaphragm, flexor digitorum superficialis, gastrocnemius, and triceps brachii muscles were collected and fixed in 10% NBF and processed as described above.

Five adult dogs were used as controls for the muscle biopsy portion of the necropsy protocol. Two dogs were intact males, a golden retriever and a German shepherd crossbred dog. Three dogs were females, a Siberian husky, a Samoyed crossbred dog, and a Springer spaniel. All 5 control dogs were free of external gross lesions. The dogs were killed with an intravenous overdose of sodium pentobarbital, and portions of the temporalis, extensor carpi radialis, flexor digitorum superficialis, tibialis cranialis, and gastrocnemius muscles were collected from each, fixed in 10% NBF, and processed similarly to muscles from the collie dogs.

Because numerous muscles that had no gross lesions did have histologic lesions, the degree of muscle involvement was evaluated histologically and categorized as minimal if one or a few inflammatory foci were present, mild if multiple distinct foci of inflammation and degeneration were present that occupied less than one-eighth of the histologic area of the muscle, moderate if one-eighth to one-half of the muscle was involved, and severe if one-half to all the muscle was involved. The degree of severity of involvement of each muscle was calculated by assigning numerical values: 0, no lesions; 1, minimal; 2, mild; 3, moderate; and 4, severe. Numbers for each muscle in all dogs (eg, all 9 longissimus dorsi muscles) were totaled to quantify the differ-

ences in severity of inflammation among muscles. Numbers for all muscles in each dog (eg, all muscles in Dog 31) were totaled for quantifying the differences in severity of myositis among the dogs. The dogs were ranked from the most to the least severely affected (from 9 to 1), on the basis of muscle lesions.

The degree of severity of skin involvement was evaluated grossly because the gross appearance of the skin was judged a more accurate measure of severity of dermatitis than a limited number of cutaneous biopsies. The dogs were ranked from the most to the least affected (from 9 to 1) on the basis of the degree of alopecia, hypopigmentation, hyperpigmentation, scaling, erosion, and crusting present.

The circulating immune complexes (CICs) in the serum that had been collected at 2-week intervals were coprecipitated from serum with equine Clq-like factor (RhC), then dissolved in ammonium acetate and quantitated in duplicate for dog IgG and in single aliquots for dog IgM, with radial immunodiffusion (RID), by the method of McDonald,[22,23] McDonald et al,[24] and Haupt et al.[18] Concentrations of CICs were expressed as micrograms per milliliter. Serum was considered to contain elevated levels of CICs if the values were 2 SD above control levels. Control sera were collected from three Doberman pinscher female dogs (7, 7, and 8.5 months of age) and a male weimaraner dog (3 months of age). Concentrations of noncomplexed dog serum IgG were also quantitated in duplicate by RID and the values expressed as milligrams per milliliter. Commercially prepared RID plates (Miles Scientific, Naperville, Ill) with rabbit antidog IgA and IgM, were used for quantitating the concentrations of IgA and IgM, respectively, in the serum of the dogs. Single aliquots of the same serum samples analyzed for CICs and IgG were analyzed for IgA and IgM, and the concentrations were expressed as milligrams per 100 ml.

Results

The 9 collie pups were free of dermatitis, myositis, and elevated levels of CICs at 6 weeks of age. The 3 German shepherd control dogs were also free of dermatitis and myositis. The collie dogs developed variably severe skin lesions between 8 and 11.5 weeks of age. The distribution, character, and progression of the dermatitis were similar to those in previously described collies with dermatomyositis.[16,17] By 12 weeks of age all collie dogs had a mild to moderate decrease in the size of temporalis muscle. In Dogs 33 and 35, the decrease in size was noted at 6 weeks of age, even though myositis was not seen histologically in two muscle samples. In 7 dogs, the general muscle mass was normal, but in the 2 most severely affected dogs (37 and 34), generalized decrease in muscle mass was noted at 20 and 28 weeks of age, respectively.

Histologic examination of the muscle samples collected at 9–12 weeks of age revealed focal areas of marked myofiber diameter variation, internal nuclei, internal myofiber laminations, and fibrosis. These changes were judged the result of scarring from previous muscle biopsy. Evidence of primary myositis was not seen. The histologic appearance of cutaneous lesions was similar to previous descriptions.[16]

Histologic examination of the muscle specimens collected at 20–23 weeks of age, from the left side where no previous surgery had been performed, revealed that 6 (31, 32, 34, 35, 36, 37) of the 9 dogs had minimal to severe myositis in the temporalis muscle. Two of these 6 dogs (34 and 37) also had mild to severe myositis in the extensor carpi radialis. One dog (33) without myositis had areas of myofiber degeneration and regeneration. The histologic appearance of the myositis was similar to previous descriptions.[16] Dog 37 had the most severe myositis and also had necrotizing vasculitis of a medium-sized muscular artery in the temporalis muscle (Figure 1).

Results of EMG and RNS studies are listed in Tables 1, 2, 3, and 4. Fibrillation potentials, positive sharp waves, and bizarre high frequency discharges were present in the collies. Abnormalities were most frequent in the muscles of mastication, followed by the muscles of distal extremities. There was no correlation of EMG abnormalities with disease severity by dog, but with the exception of the tongue, there was correlation of EMG abnormalities and the disease severity within muscles. The incidence of abnormalities was highest at 6 weeks of age, followed by 7.5 months and 20–23 weeks of age. Fibrillation potentials were present also in the 7-week-old weimaraner–Doberman pinscher crossbred pups, but there were no abnormalities in German shepherds. At 7.5 months there were decremental responses in the

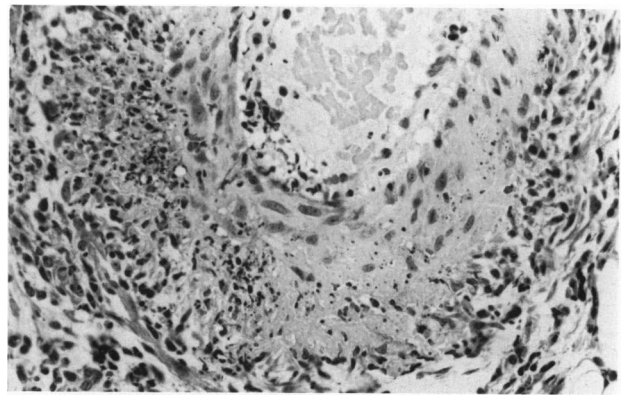

Figure 1—Vasculitis of medium-sized artery in temporalis muscle of severely affected Dog 37. Note necrosis. (H&E, ×200)

Table 1—Results of Electromyography in Collies

Muscles	31				32				33				34				35				36				37				38				39				Total number of changes
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
Pelvic Limb																																					
Interosseous	.	.	.	.	.	.	.	.	.	.	.	f	.	.	.	pf	.	f	.	.	.	.	f	.	.	.	p	.	.	.	f	.	.	.	f	.	8
Digital flexors	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0
Tibialis cranialis	f	.	.	.	.	.	.	.	f	.	.	.	.	.	.	p	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
Gastrocnemius	f	.	.	.	f	.	.	.	f	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	f	.	.	.	4
Semimembranosus and semitendinosus	.	.	.	.	.	.	.	.	f	.	.	.	.	.	.	.	f	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
Quadriceps	.	.	.	.	.	.	.	.	f	.	.	.	.	.	.	.	f	.	.	.	f	.	.	.	.	.	.	.	.	.	.	.	f	.	f	.	5
Gluteal	.	.	.	.	.	.	.	.	.	f	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
Paraspinal																																					
L_3–S_2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0
L_4–T_2	f	.	.	.	.	.	.	.	.	.	.	.	f	.	f	.	f	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	f	.	.	5
C_5–T_1	.	.	.	.	.	.	.	.	f	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	f	.	.	.	.	.	.	.	2
C_1–4	.	.	.	.	f	.	.	.	.	.	.	.	.	.	.	.	f	.	.	.	f	.	.	.	f	.	.	.	.	.	.	.	.	f	.	.	5
Thoracic																																					
Interosseous	f	.	.	.	f	.	.	.	.	f	.	f	.	.	.	f	.	f	.	.	f	.	.	.	.	.	.	f	.	.	p	.	.	.	.	f	10
Extensor carpi radialis	f	.	.	.	f	f	.	.	f	f	.	f	f	.	.	.	.	.	.	.	f	.	.	.	f	.	p	pf	.	.	.	.	f	.	.	p	14
Digital flexors	f	.	.	.	.	.	.	.	f	.	.	.	f	.	.	.	f	.	.	.	f	.	.	.	pf	.	.	.	.	.	f	.	f	.	.	.	9
Triceps brachii	f	.	.	f	.	.	.	.	f	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	f	.	4
Biceps brachii	f	.	.	.	.	.	.	.	f	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	f	.	.	f	.	.	.	4
Deltoideus	.	.	.	.	.	.	.	.	f	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
Infraspinatus	.	.	.	.	f	.	.	.	f	.	.	.	f	.	.	.	f	.	.	.	.	.	.	.	.	.	pf	.	.	.	.	.	f	.	.	.	7
Supraspinatus	f	.	.	.	.	.	.	.	f	.	.	.	f	.	.	.	f	.	.	.	.	.	f	.	.	.	.	.	.	f	.	.	.	.	.	.	6
Head																																					
Temporalis	pf	f	f	f	f	.	f	.	pf	.	.	f	f	.	f	.	f	.	.	f	f	.	p	.	f	.	f	pf	f	.	f	f	f	f	f	f	27
Masseter	f	.	f	f	f	.	f	f	f	.	.	f	f	.	.	f	f	.	f	f	f	.	.	f	pf	.	f	pf	f	.	f	f	pf	.	p	.	26
Tongue	.	f	.	.	.	.	f	f	f	.	.	f	f	f	p	p	f	f	.	bf	f	.	pf	.	f	.	.	.	f	.	.	f	f	f	f	p	23
Facial	.	.	.	b	.	.	.	.	f	.	.	f	.	.	.	f	pf	.	.	b	.	.	.	b	.	.	f	b	.	.	.	b	.	.	.	.	11
Total number of changes	12	2	2	4	7	1	3	2	17	3	0	7	8	1	3	7	12	3	1	5	8	0	5	2	8	0	7	8	4	2	5	4	10	4	6	4	177

1, 6 weeks of age; 2, 9–12 weeks of age; 3, 20–23 weeks of age; 4, just before 7.5 months of age; f, fibrillation potentials; p, positive sharp waves; b, bizarre high frequency discharges.

Table 2—Results of Electromyography in Weimaraner–Doberman and German Shepherd Control Pups

Muscles	Weimaraner–Doberman				German shepherd			Total number of changes
	M1	M2	M3	F1	M1	F1	F2	
Pelvic limb								0
Interosseous	.	.	.	.	.		.	0
Digital flexors	.	.	.	.	.	.	.	0
Tibialis cranialis	.	.	.	.	.	.	.	0
Gastrocnemius	.	.	.	.	.	.	.	0
Semimembranosus semitendinosus	.	.	.	.	.	.	.	0
Quadriceps	.	.	.	.	.	.	.	0
Gluteal	.	.	.	.	.	.	.	0
Paraspinal								
L_3–S_2	.	.	.	.	.	.	.	0
L_4–T_2	.	.	.	.	.	.	.	0
C_5–T_1	.	.	.	.	.	.	.	0
C_1–C_4	.	.	.	.	.	.	.	0
Thoracic limb								
Interosseous	.	.	.	.	.	.	.	0
Extensor carpi radialis	.	.	.	.	.	.	.	0
Digital flexors	f	.	.	.	.	.	.	1
Triceps brachii	.	.	.	.	.	.	.	0
Biceps brachii	f	.	.	.	.	.	.	1
Deltoideus	.	.	.	.	.	.	.	0
Infraspinatus	.	.	.	.	.	.	.	0
Supraspinatus	.	.	.	f	.	.	.	1
Head								
Temporalis	.	f	f	.	.	.	.	2
Masseter	.	f	.	.	.	.	.	1
Tongue	.	f	.	.	.	.	.	1
Facial	.	.	.	.	.	.	.	0
Total number of changes	2	3	1	1	0	0	0	7

f, fibrillation potentials; p, positive sharp waves; M1, Male 1; M2, Male 2; M3, Male 3; F1, Female 1; F2, Female 2.

Table 3—Repetitive Nerve Stimulation Studies in Dogs at 7.5 Months of Age

Nerve	Dog (motor nerve conduction velocity in meters/sec)								
	31	32	33	34	35	36	37	38	39
Sciatic–Tibial	64	68.6	66.7	67.7	67.3	66.9	72	62.5	66.6
Distal–Tibial	47.5*	57.1	54.1	53.7	51.3	52.6	52.9	51	55.6
Ulnar	65	62.9	63.5	66.9	62	57.2	57.7*	56	61.2

* Denotes when near-nerve temperature was 36 C. Near-nerve temperature was between 37 and 38 C in all other cases.

Table 4—Repetitive Nerve Stimulation Studies in Dogs at 7.5 Months of Age

Nerve	Percent Decrement in Response to Repetitive Nerve Stimulation for Dog								
	31	32	33	34	35	36	37	38	39
Distal–Tibial									
5 Hz	21	13	10	None	12	25	None	None	9.6
10 Hz	28	30	25	None	29	31	None	22	19
Ulnar									
5 Hz	None	20	None	None	None	None	None	None	None
10 Hz	None	29	None	None	None	None	None	None	None

Table 5—Distribution of Gross Cutaneous and Muscular Lesions in Collie Dogs with Dermatomyositis

	Dog								
	31	32	33	34	35	36	37	38	39
Temporalis	Bilateral anterior + superficial Scar	0 Scar	0 Scar	Anterior + superficial	0 Scar	0 Scar	Anterior + superficial	0 Scar	0 Scar
Extensor carpi radialis	0	Scar	Scar	Scar	0	0	0	+	Scar
Extensor digitorum communis	0	0	0	0	0	Scar	0	0	0
Extensor front legs	0	0	0	+	0	0	+	0	0
Extensor hind legs	0	0	0	0	0	0	+	0	0
Flexor digitorum superficialis	0	0	0	0	0	0	+	0	0
Gastrocnemius	0	0	0	0	0	0	+	0	0
Longissimus	0	0	0	0	0	0	Superficial +	0	0
Iliocostalis	0	0	0	0	0	0	Superficial +	0	0
Sternohyoideus	0	0	0	0	0	0	+	0	0
Triceps brachii	0	0	Deep +	0	0	0	0	0	0
Tibialis cranialis	0	0	0	+	0	+	0	0	0
Extensor digitorum longus	0	0	0	+	0	0	0	0	0
Extensor digitorum lateralis	0	0	0	0	0	0	0	Scar	0
Masseter	0	0	0	0	0	0	+	0	0
Face	Alopecia Hyperpigment Erythema Crusts	Mild Alopecia Hyperpigment	Erythema Alopecia Hyperpigment Hypopigment	Erythema Alopecia Hyperpigment Hypopigment	Erythema Alopecia Hyperpigment	0	Erythema Alopecia Crusts Hyperpigment Hypopigment	Erythema Alopecia Hyperpigment Hypopigment	Erythema Alopecia Hyperpigment

distal-tibial or ulnar nerves of collies 31, 32, 33, 35, 36, 38, and 39 (Table 4).

Two biopsies of skin of the ear were collected for immunofluorescence from areas of erythema, crusting, or vesiculation between 9 and 12 weeks of age in each of the nine dogs. Immunofluorescence studies for the deposition of IgG and C3 were negative.

At 7.5 months of age the 9 dogs were euthanized by intravenous injection of an overdose of sodium pentobarbital and were necropsied. The distribution and variable severity of dermatitis are presented in Table 5, and were similar to those in previous reports.[16,17] Briefly, areas of alopecia, hyperpigmentation, hypopigmentation, erythema, scaling, crusting, and erosions were present on the face, ears, elbows, carpi, stifles, tarsi, feet, and tip of tail. The ranking of the dogs with respect to the degree of severity of grossly visible skin lesions, from most severe to least severe, was $37 \geqslant 34 > 32 > 31 \geqslant 38 = 39 > 33 > 35 = 36$ (Table 6). Histologically, in the mildly affected dogs, mild hyperkeratosis and acanthosis were present. A few scattered or clustered lymphocytes, macrophages, plasma cells, mast cells, and eosinophils were in the papillary dermis, perifollicularly or perivascularly. Some macrophages contained melanin, and a few basal cells were pyknotic or vacuolated. A few hair follicles were slightly dilated. Occasionally amphophilic, homogeneous material was present in the dermis. In moderately to severely affected dogs, the inflammation was qualitatively similar to that of the mildly affected dogs but was more extensive. Der-

Table 5—*Continued*

	Dog								
	31	32	33	34	35	36	37	38	39
Ears	Scales	Scales	Scales Alopecia Crusts	Scales Alopecia Erosion Crusts Erythema	Crusts Ulcer	Alopecia Scales	Erythema Alopecia Crusts Erosion Scales Hypopigment	Scales	Scales
Elbows	0	0	0	Crusts Ulcer	0	Crusts Ulcer	Alopecia	0	0
Carpi	Erythema Alopecia Crusts Erosion	Alopecia	0	Erythema Alopecia Crusts Erosion	0	Erythema Alopecia	Erythema Alopecia Crusts Erosion	Erythema Alopecia	0
Stifles	Crusts Erosion	0	0	Alopecia Scales	0	0	Alopecia Scales	Mild Alopecia Scales	0
Tarsi	Erythema Alopecia Scales	0	0	Erythema Alopecia Crusts Erosion	0	0	Scales Erythema Alopecia Crusts Erosion	0	0
Feet	Crusts Erosion Scales	0	0	Erythema Alopecia Crusts Erosion	0	0	Erythema Alopecia Crusts Erosion	0	0
Tail tip	Erythema Alopecia Scales	Alopecia Scales	Alopecia Scales	Erythema Alopecia Scales Crusts	Alopecia Scales Hyperpigment	Alopecia Scales	Erythema Alopecia Scales Crusts	Alopecia Scales	Alopecia Scales
Severity of dermatitis (1 to 9)	6	7	3	8	2	1	9	5	4
Body wt (kg)*	22.2	24	22	21.3	25.4	27.2	18.6	30.4	30
Sex	F	F	F	F	M	M	M	M	M

+, lesion; 0, no lesion; *, at 7.5 months; Scar, result of previous surgery.

mal macrophages also frequently contained melanin, dermal fibrosis was present, and many adnexal structures were small or absent. Ulcers were present in some dogs. The two most severely affected dogs, 37 and 34, had arteritis (Figure 2). Arterial walls were thickened by ovoid to spindle-shaped cells in an eosinophilic fibrillar matrix. The arterial lumens were frequently narrowed, and nuclei in the walls were pyknotic. *Demodex canis* were present in follicles in Dogs 37 and 34, and folliculitis was present.

Although grossly abnormal muscles were seen (Table 5), many muscles with histologic lesions were grossly normal. In general, the more superficial protions of muscles and the muscles of the distal portions of the extremities of the body were more severely affected. The anterior and superficial portions of the temporalis muscle were reduced in size and were pale tan and soft in Dogs 31, 34, and 37. In the most severely affected dog (37), the distribution of the muscle lesions was more generalized. At least 1 dog had pale tan areas, especially superficially, in one of the following muscles or muscle groups: temporalis, front or hind leg extensors below the elbow or stifle, flexor digitorum superficialis, gastrocnemius, longissimus dorsi, iliocostalis, sternohyoideus, tibialis cranialis, and masseter. The triceps brachii of one dog had a pale tan area more deeply located. The temporalis and extensor carpi radialis muscles had depressed, firm tan areas in areas of previous surgery, which were judged to be fibrosis (scarring). In Dog 36 the extensor digitorum communis, and in Dog

Table 6—Mean Concentration of Circulating Immune Complexes and Immunoglobulins in 13 Sequential Serum Samples From the 9 Dogs and Comparison With the Degree of Dermatitis and Myositis

Dog	CICs (μg/ml)	IgG (mg/ml)	IgA (mg/100 ml)	IgM (mg/100 ml)	Severity of lesions*		
					Dermatitis	Myositis	Combined
31	85.2 ± 8.8	17.4 ± 1.2	18.1 ± 1.5	178 ± 35	6	3	6
32	67.2 ± 3.4	12.0 ± 0.6	16.4 ± 1.1	164 ± 23	7	7	7
33	30.2 ± 2.6	8.5 ± 0.8	25.5 ± 5.9	146 ± 31	3	2	2
34	70.5 ± 6.8	12.0 ± 0.8	16.0 ± 1.5	117 ± 16	8	8	8
35	74.4 ± 6.3	11.3 ± 1.0	21.9 ± 4.0	206 ± 45	2	5	4
36	34.3 ± 3.5	7.1 ± 0.5	16.9 ± 1.4	148 ± 19	1	6	3
37	140.3 ± 18.5	25.2 ± 3.2	20.6 ± 2.1	121 ± 13	9	9	9
38	38.8 ± 3.7	9.8 ± 0.9	21.3 ± 4.6	91 ± 13	5	4	5
39	25.9 ± 3.2	10.1 ± 0.9	21.2 ± 3.7	98 ± 17	4	1	1

* Most severe, 9; least severe, 1.
Concentrations are expressed as the mean ± SEM.

38 the extensor digitorum lateralis also had areas of fibrosis judged to have resulted from previous surgical biopsies in the area of the extensor carpi radialis. No gross lesions were seen in the muscles of the 5 adult control dogs.

The histologic changes in muscle were similar to those described previously.[16] Briefly, a variable number of lymphocytes, macrophages, plasma cells, and fewer neutrophils and eosinophils were present in endomysium and perimysium, and surrounding small blood vessels. In some muscles, myofibers were replaced by these cells. Myofiber changes included diameter variation, increased acidophilia, vacuolization, and fragmentation. In addition, scattered myofibers were basophilic and frequently had large vesicular nuclei with a large prominent nucleolus. Other nuclei were internalized. The temporalis muscles of Dogs 34 and 37 had smaller myofibers at the periphery of the fascicles, but generally there was no distinct perifascicular atrophy. Also, there were lymphocytes within the tunica adventitia and media of an artery in the temporalis muscle of Dog 37. The inflammation was so extensive in this muscle that similar inflammatory cells were present also within and around a small nerve in the perimysium. The temporalis and masseter muscles had small nerves with fewer axons than expected, and lightly eosinophilic to amphophilic fibrils were present in epineurium and endoneurium.

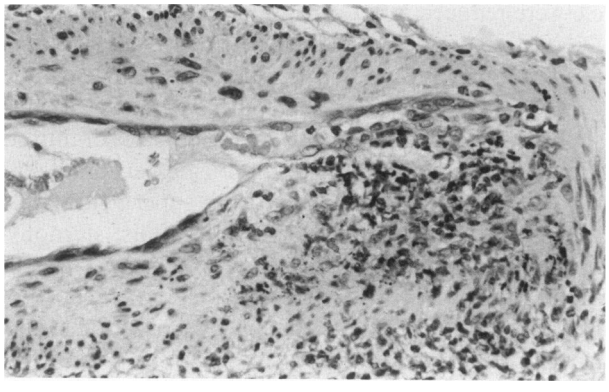

Figure 2—Vasculitis of artery in skin of severely affected Dog 37. Note localized thickening of wall and pyknotic nuclei. (H&E, ×200)

The degree of severity of histologic muscle lesions, from the dog most severely affected to the dog least severely affected, was 37⩾34>32>35=36>31=38>33>39 (Tables 6 and 7). The 3 dogs with the most severe skin changes (37, 34, 32) also had the most severe muscle lesions (Tables 5 and 6). There was less correlation between the degree of dermatitis and myositis in the remaining 6 dogs.

Histologically, 1 control dog, the German shepherd male, had several lymphocytes and macrophages clustered about a fragmented myofiber in the extensor carpi radialis muscle. A similar focus, but without a fragmented myofiber, was present in the flexor digitorum superficialis muscle. Other histologic lesions were not observed in muscles of control dogs (Table 8).

The CIC evaluations revealed that all dogs were initially free of elevated levels of CICs. The levels of CICs began to rise before or concurrent with the onset of dermatitis in collie dogs (Figure 3). The CIC levels at 2-week intervals are presented in Table 6. Levels of CICs began to rise between 6 and 10 weeks of age, dermatitis began between 8 and 11.5 weeks, and myositis was not detected between 9.5 and 12.5 weeks but was present at 21 weeks of age. The CICs tended to peak between 14 and 18 weeks of age except in Dog 37 (Figure 4). In order to correlate the degree of severity of dermatomyositis and elevated levels of CICs, we determined the degree of severity of both skin and muscle lesions by adding the numerical value of severity of skin lesions and muscle lesions for each dog and dividing by 2. The degree of severity of dermatomyositis from most to least severe was 37⩾34>32>31=38>35=36>33=39. In the mildly affected dogs the CIC concen-

Table 7—Histologic Evaluation of Myositis in Dogs with Dermatomyositis

Dog	Longissimus	Hyopharyngeus or thyropharyngeus	Intercostal	Temporalis	Masseter	Biceps femoris	Diaphragm	Flexor, digitorum superficialis	Gastrocnemius	Triceps brachii	Degree of severity per dog
31	0	0	0	Mild to moderate	0	Minimal	0	0	0	Minimal	5
32	0	Minimal	0	Mild	Mild	0	0	Minimal to mild	0	0	7
33	0	0	0	Mild	0	Minimal	0	Minimal	0	0	4
34	0	Minimal to mild	0	Moderate	Minimal	Minimal	0	Minimal	0	Minimal	9
35	0	Minimal to mild	Minimal	Minimal	Minimal	0	0	Minimal	0	0	6
36	0	Minimal	0	Mild	Minimal	0	0	0	Minimal	Minimal	6
37	Mild	Mild	Moderate	Severe	Severe	0	0	Moderate to severe	Mild	Minimal	22
38	0	Minimal	0	Mild	0	0	0	Minimal	0	Minimal	5
39	0	0	0	Minimal	0	0	0	Minimal	0	0	2
Degree of muscle severity	2	9	4	20	9	3	0	11	3	5	

0, no lesions.

trations, after peaking at 14 to 18 weeks of age, tended to drop to normal values, the CICs in moderately affected dogs tended to remain at elevated levels, and the CICs in the most severely affected dog (37) continued to rise (Figure 4). The highest CIC level, reached by Dog 37, was 238 μg/ml (control = 30 μg/ml). The mean CIC concentrations from 13 sequential serum samples from each of the 9 dogs is presented in Table 6. The ranking of the dogs from the greatest to least concentration was 37⋙31>35>34>32⋙38>36>33>39 (Table 6). The concentration of IgM in the CICs was less than 10 μg/ml in each sample from each collie dog. The concentration of IgM in CICs in the serum of control dogs ranged from undetectable (about 2 μg/ml) to 15 μg/ml. Except for 1 dog (35), the severity of dermatomyositis correlated positively with higher CICs.

Table 8—Histologic Evaluation of Myositis in Control Dogs

Dog	Temporalis	Extensor carpi radialis	Flexor digitorum superficialis	Cranialis tibialis	Gastrocnemius
Control 1	0	0	0	0	0
Control 2	0	Normal to minimal	Normal to minimal	0	0
Control 3	0	0	0	0	0
Control 4	0	0	0	0	0
Control 5	0	0	0	0	0

0, no lesions.

Serum IgG levels were also determined (Figure 5). The ranking of the dogs on the basis of the mean IgG concentration in the serum from the highest concentration to the lowest was 37⋙31>32=34>35>39=38>33>36 (Table 6). In general, there was a strong association between high IgG and high CIC levels (Figures 4 and 5). There were no differences from controls in IgM and IgA levels, and the levels of these immunoglobulins did not correlate with the degree of severity of dermatomyositis or concentrations of serum CICs or IgG (Table 6) (Figures 6 and 7).

Discussion

Dermatomyositis is considered by some to be an autoimmune disease,[11] a connective tissue disease,[5] and an inflammatory myopathy.[25] Polymyositis, also an inflammatory myopathy, may be a variant of dermatomyositis in which skin lesions are not present.[26] There is evidence supporting cell-mediated[12] and humoral mechanisms[13] in the pathogenesis of dermatomyositis. Immune complex deposition has also been implicated as a mediator of the disease, especially in children.[27,28] Evidence implicating immune complexes is based on observations that vasculitis[29] often caused by immune complex deposition has been seen in dermatomyositis; dermatomyositis has developed subsequent to a variety of viral infections, immunizations, and drug therapy[30–32]; immune complexes have been identified in some patients with dermatomyositis[27,14]; complement

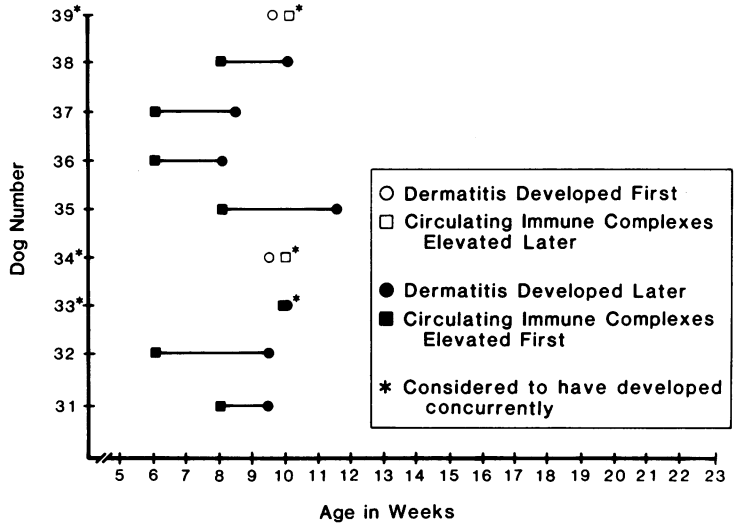

Figure 3—Comparison of onset of CICs with onset of dermatitis in Dogs 31–39.

activation has developed in some patients[33]; and immunofluorescent examination of muscle has revealed the deposition of immune complexes in small vessels.[28]

In a previous report on dogs with dermatomyositis, the most prominent immunologic finding was elevated levels of CICs, but because the assays were performed on serum obtained during the chronic phase of the disease, conclusions regarding the causative nature of the immune complexes could not be made.[18] In the dogs in this study, levels of CICs began to rise above control levels before dermatitis, myositis, or vasculitis were detected, which suggests that the immune complexes initiated the inflammation rather than resulted from it. The levels of CICs correlated with the severity of the dermatitis and myositis in all but one dog; and, in mildly affected dogs, levels of CICs decreased to normal levels as the disease resolved, indicating that the elevated CIC levels were temporally associated with the lesions. Another factor implicating immune-complex-mediated disease in canine dermatomyositis was vasculitis in two dogs, both of which had high levels of CICs. High concentrations of serum IgG were also present in the affected dogs and correlated generally with the severity of dermatomyositis and the concentrations of CICs. The immunoglobulin component of the CICs was IgG.

In a previous report, immunofluorescence studies failed to show deposition of immunoglobulin or complement in cutaneous or muscular tissues in dogs with

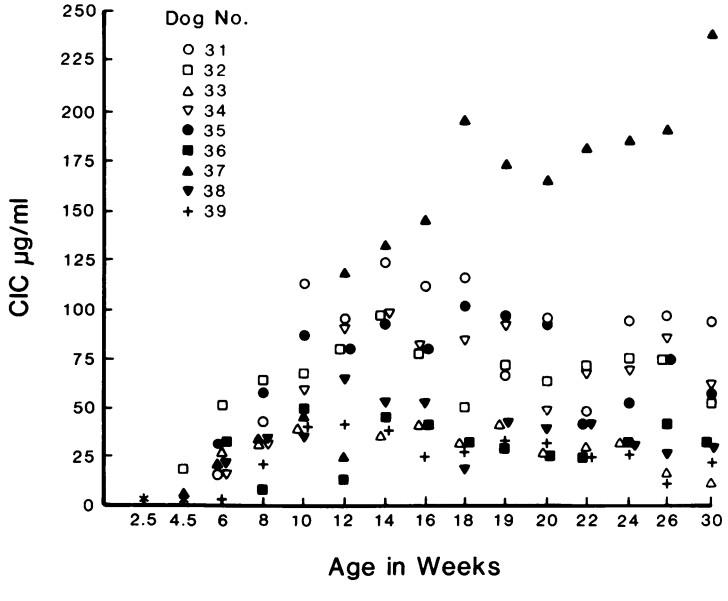

Figure 4—Comparison of CIC levels in Dogs 31–39 from 2.5 to 30 weeks of age. *Asterisks* refer to multiple dogs with the same concentrations of CICs or of immunoglobulins at the same point in time.

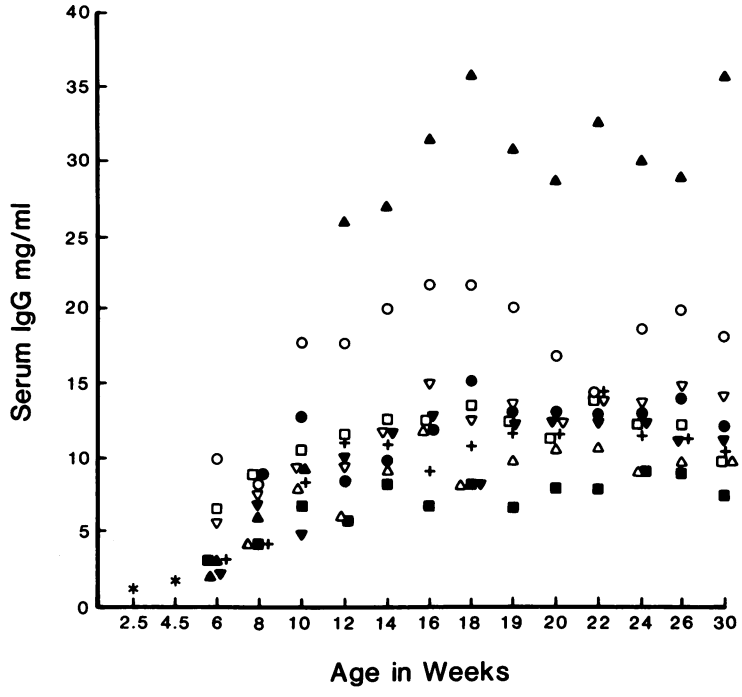

Figure 5—Comparison of IgG levels in Dogs 31–39 from 2.5 to 30 weeks of age. *Asterisks* refer to multiple dogs with the same concentrations of CICs or of immunoglobulins at the same point in time.

dermatomyositis.[18] Immunofluorescence of two cutaneous samples from areas of acute dermatitis in each of the 9 collies in this report was also negative. In human dermatomyositis, immunologic techniques applied to skin are usually negative.[34] Positive immunofluorescence of muscle has been reported in persons with dermatomyositis in the sarcolemmal basement membranes, within muscle fibers, and within vessels.[28,35,36] However, lack of consistent staining with direct immunofluorescence in patients with rheumatic diseases makes the use of this technique of limited value in diagnosis.[37] It has also been shown that detection by immunofluorescence of immune complexes in the skin is time-dependent, and lesions less than 18 hours old will yield positive results, but older lesions may be negative.[38] Recently a study on diagnosis of canine autoim-

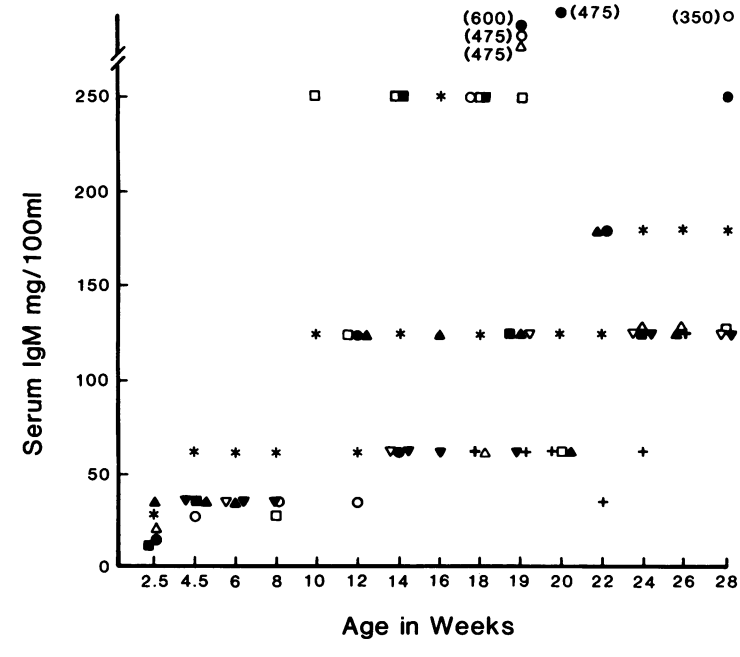

Figure 6—Comparison of IgM levels in Dogs 31–39 from 2.5 to 30 weeks of age. *Asterisks* refer to multiple dogs with the same concentrations of CICs or of immunoglobulins at the same point in time.

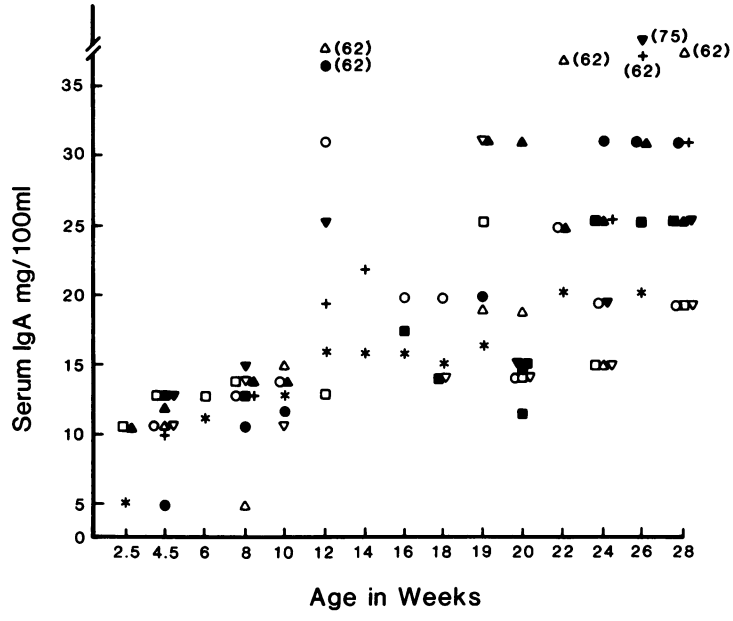

Figure 7—Comparison of IgA levels in Dogs 31–39 from 2.5 to 30 weeks of age. *Asterisks* refer to multiple dogs with the same concentrations of CICs or of immunoglobulins at the same point in time.

mune skin disease, not including dermatomyositis, revealed positive direct immunofluorescence in only 52% of the cases.[39] Because of variable results, it was recommended that accurate diagnosis of canine autoimmune skin disease requires evaluation of a combination of clinical, histologic, and immunopathologic factors.[39] Therefore, even though the immunofluorescent staining of muscle and skin for IgG and C3 in dogs with dermatomyositis was negative, immune complexes may have played a role in pathogenesis of the disease. The immune complexes may have been present before biopsies were performed but were removed from the tissues by the time of biopsy.

There is a possibility that the distribution of lesions in canine dermatomyositis may be related to the lower body temperature in peripheral regions of the body. The distribution of lesions in dogs with dermatomyositis followed the pattern of haircoat coloration in Siamese cats, which is known to be related to temperature.[40-42] In dogs with dermatomyositis the cutaneous lesions were most severe on the ears, face, and distal extremities, including the tip of the tail. The distribution of the canine muscle lesions paralleled the cutaneous lesions. When the muscle lesions in dogs were more generalized, only the superficial portions of the muscles were involved, not the deep portions. Temperature is known to modulate the immune reaction in a variety of ways. Elevated body temperature (fever) has been shown to be beneficial in eliminating infections[43] and in reducing deaths due to infections in animals.[44-46] Increased bactericidal capacity by human neutrophils has been demonstrated *in vitro* when incubation temperatures are increased.[47] Increased temperature also enhances the antiviral activity of human interferons.[48] Lymphocytes incubated with T-cell mitogens *in vitro* at elevated temperatures have enhanced activity as determined by incorporation of ^{3}H-thymidine into DNA.[47,49] The modulation of the immune system by temperature can be generalized, as in fever, or localized. In spontaneous and experimental infections with *Mycobacterium leprae*, lesions develop in cooler regions of the body.[50-52] The armadillo is the only nonprimate known to show development of disseminated lesions of leprosy following inoculation,[53] and these animals have a low body temperature (32–35 C) that is comparable to the cooler regions of the human body.[54] Lymphocytes from armadillos have enhanced transformation to nonspecific (PHA) and specific (Lepromnin) mitogens when incubated at higher (37 C) rather than lower (28–33 C) temperatures, thus implicating temperature as a component in the modulation of the cell-mediated immune response.[55] It was recently reported that sooty mangabey monkeys developed disseminated lepromatous leprosy, but the lesions were confined to skin, peripheral nerves, nasal mucosa, eyes, testes, and peripheral lymph nodes, with minimal internal involvement.[56] Another factor that may influence lesion distribution in the dogs is cryoglobulin, an immunoglobulin that precipitates or gels when exposed to cold temperatures. Cryoglobulins consist of immune complexes and have been associated with a variety of autoimmune and systemic connective tissue diseases in which CICs play a role.[57]

Brouet et al[58] found that in two-thirds of patients with cryoglobulinemia, cutaneous symptoms were the major presenting problem. Even though dogs with dermatomyositis have high levels of CICs, it is not known whether cryoglobulins are present. Even if cryoglobulins are not present in affected dogs, reduced peripheral body temperature could influence deposition of immune complexes. Reduced temperature reduces cutaneous blood flow, which increases contact time between immune complexes and the endothelium, thus increasing the possibility of immune complex deposition.[59] Other factors shown to enhance immune complex deposition that may be significant in canine dermatomyositis include increased local pressure and presence of previous lesions.[59] The lesions of the elbows, hocks, stifles, carpi, and tarsi in dogs could be due to immune complex deposition in areas of trauma over these bony prominences. The presence of preexisting dermatitis may also predispose to immune complex deposition in muscle subjacent to cutaneous inflammation.

There are similarities in the distribution of skin lesions in humans and those observed in dogs with dermatomyositis. In man the cutaneous rash is most severe over the upper eyelids, forehead, cheeks, neck, and extensor surfaces of extremities, including knuckles, elbows, knees, and ankles.[5] The distribution of the myositis in dogs with dermatomyositis, however, differed from that in man. In man the muscle lesions are in the more proximal muscles, especially the neck, shoulder, and pelvic girdles.[5] This difference in lesion distribution may indicate a difference in pathogenesis of human and canine dermatomyositis.

The results of EMGs were similar to those of previous reports of dermatomyositis in collies.[17,60] Mild decremental responses with RNS studies have also been reported.[17] There was no correlation between abnormalities of EMG or results of RNS studies and disease severity; but, with the exception of the tongue, there were more alterations in the EMGs of more severely inflammed muscles. Some of the EMG abnormalities occurred when there was no histologic evidence of myositis, which may indicate that there was a biochemical predisposition to myositis before histologic evidence of disease became apparent. Alternatively, myositis may have been present but undetected. Another possible explanation is that normal canine neonatal muscle may exhibit fibrillation potentials during needle electromyographic procedures. It has been shown, for example, that normal human infants have needle EMG abnormalities.[61] Each of the 7-week-old weimaraner–Doberman pinscher crossbred pups had fibrillation potentials, indicating this may be true in dogs as well. Muscle biopsies were not performed on the weimaraner–Doberman control pups to completely rule out the presence of myositis, but there was no clinical or historical evidence to indicate myositis was present, and the likelihood of myositis in the pups was remote. The histologic evidence of alterations of a few small peripheral nerves in some dogs with dermatomyositis suggests there may be a neurologic role in some of the abnormalities detected by RNS studies. It is possible that inflammatory changes in peripheral nerves, possibly including the myoneural junction, incorporated within areas of myositis may result in defects of myoneural transmission.

Vaccination has also been associated with the development of dermatomyositis.[31] In the collies in this study the initial vaccination for canine distemper, adenovirus, and leptospirosis preceded development of dermatitis by several days. In a previous report[17] collies developed dermatitis between 7 and 11 weeks of age and were vaccinated for canine distemper, adenovirus, parainfluenza, leptospirosis, and parvovirus at 8 and 12 weeks of age, indicating that in at least some of the dogs dermatitis developed before vaccination. The vaccination and subsequent development of dermatitis was probably coincidental. Additional studies regarding the possible role of viruses or vaccination in the development of canine dermatomyositis need to be performed.

Because the development, severity, and progression of dermatomyositis in collies were strongly correlated with elevated levels of CICs and serum IgG, it is suggested that CICs and IgG are important in the pathogenesis of canine dermatomyositis. The cause or causes of the elevations of CIC and IgG levels are unknown.

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