

Acquired Myasthenia Gravis

Selective Involvement of Esophageal, Pharyngeal, and Facial Muscles

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Serum samples from 152 dogs with a clinical diagnosis of idiopathic megaesophagus without detectable generalized muscle weakness were tested for the presence of antibodies to acetylcholine receptors by immunoprecipitation radioimmunoassay. Positive serum antibody titers (mean, 3.1 nmoL/L; range, 0.77–30 nmoL/L; reference values < 0.6 nmoL/L) were found in 40 dogs (26%), with German Shepherd dogs (8/25, 32%) and Golden Retrievers (7/20, 35%) having a greater percentage of positive submissions. By immunocytochemical methods, localization of immune complexes at the neuromuscular junction after incubation of serum with normal canine muscle was documented in an additional 17 cases (11% of all samples submitted) that did not have increased antibody titers to acetylcholine receptors. Of the 40 seropositive dogs, 17 (48%) had a clinical improvement or remission of clinical signs associated with decreasing AChR antibody titers. Idiopathic megaesophagus has been associated with a poor prognosis; however, this study demonstrates that a large percentage of the dogs have myasthenia gravis and that with supportive treatment, the clinical signs may improve or resolve. (*Journal of Veterinary Internal Medicine* 1990; 4:281–284)

MYASTHENIA gravis (MG) is a disorder of neuromuscular transmission in which autoantibodies against nicotinic acetylcholine receptors (AChRs) at the neuromuscular junction result in a reduction of AChRs and muscle weakness.¹ The diagnosis of MG is based on demonstration of serum autoantibodies to muscle AChRs. This is objective and quantitative, and proves an autoimmune response to AChRs, as opposed to other

causes of muscle weakness such as an autoimmune response to another component of the neuromuscular junction. Subjective methods of evaluation of muscle weakness and fatigability by anticholinesterase testing or electrophysiologic responses may provide a presumptive diagnosis; however, they may give false negative or false positive results.²

In human MG, both a generalized weakness and a focal or localized weakness in which signs are limited to the extraocular muscles have been described.¹ Serum autoantibodies to AChRs can be found in 85% to 90% of those patients showing generalized signs of muscle weakness thought to be the result of MG and in 50% to 70% of those patients with strictly the ocular form.

While generalized canine acquired MG has been well documented,^{3–5} a focal form in which megaesophagus with regurgitation was the principal clinical sign has only recently been recognized. In one study, 18 of 48 dogs that had been diagnosed as having idiopathic megaesophagus were found to have elevated antibody titers to AChRs (VA Lennon, personal communication, 1989). Some dogs with this focal form of MG also had weakness involving the pharyngeal, laryngeal, and/or facial muscles. Megaesophagus in dogs, like ocular MG, may be a focal manifestation of a mild, generalized autoim-

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mune response to AChRs, which is clinically evident due to the peculiarities of local musculature. The presence of striated muscle in canine, but not human, esophagus subjects dogs to the possibility of esophageal dysfunction and subsequent potentially fatal aspiration pneumonia when transmission at striated muscle synapses is impaired.

In this study we describe the results of serologic testing of 152 dogs with idiopathic megaesophagus, pharyngeal paralysis, and/or decreased palpebral reflexes for the presence of autoantibodies against AChRs. Additionally, it was possible to follow the long-term clinical course of these disorders in those patients with antibodies to AChR.

Materials and Methods

Dogs With Megaesophagus

Serum samples from 152 dogs with clinical signs of regurgitation and radiographic evidence of esophageal dilation were submitted for determination of AChR antibody titers (57 intact males, 22 castrated males, 23 intact females, and 50 spayed females). Some of the dogs also had pharyngeal weakness and decreased palpebral reflexes. Generalized muscle weakness was not recognized in any case.

Purification of Canine AChR

For use as antigen to detect the presence of autoantibodies in canine serum, AChR was solubilized from muscle membranes of near-term canine fetuses in 2% Triton* X-100 and a crude extract prepared as previously described.⁶ The concentration of AChR was determined and expressed as moles of ¹²⁵I- α -bungarotoxin (α -Bgt) binding sites per liter of serum.

Immunoprecipitation Radioimmunoassay

The diagnosis of MG was made by immunoprecipitation radioimmunoassay as previously described.⁵ Titers were expressed as moles of ¹²⁵I- α -Bgt binding sites per liter of serum. For the determination of the titer of autoantibodies directed to the α -Bgt binding site on the AChR, modifications in the assay were made as previously described.⁵

Immunocytochemical Localization of Immune Complexes

For the demonstration of circulating antibodies against neuromuscular junction determinants (e.g., AChRs), serum was incubated with normal canine muscle and immune complexes were detected by an immunocytochemical method employing staphylococcal protein A

conjugated to horseradish peroxidase as previously described.⁷

Results

The total number of dogs and breeds affected with idiopathic megaesophagus, as well as the prevalence of focal MG, is summarized in Table 1. Twenty-six percent (40/152) of the dogs had serum AChR antibody titers greater than 0.6 nmol/L, a value previously determined to be diagnostic of MG.⁵ The mean serum titer observed in this study was 3.1 nmol/L (range, 0.77–30 nmol/L).

Numerous breeds of dogs had esophageal dilatation associated with antibodies to AChR. Golden Retrievers (7/20, 35%), German Shepherd dogs (8/25, 32%), and Labrador Retrievers (4/16, 25%) were the most prevalent breeds affected; however, our data does not necessarily reflect their respective frequencies in the population at risk for megaesophagus. These same breeds are also highly represented in generalized MG. Of 145 dogs in which we have detected antibodies to AChR, 16 (11%) were Golden Retrievers, 35 (24%) were German Shepherd dogs, and nine (6%) were Labrador Retrievers (Shelton, unpublished data, 1989). Of 91 dogs of 43 other breeds, 21% also had antibodies to AChR. Age of onset was bimodal, as in generalized MG, with a younger group of dogs having an onset at two to four years of age and an older group with an age of onset of 9 to 13 years (Fig. 1). The prevalence of increased titers was not significantly different between sexes.

Using immunocytochemical methods, immune complexes were localized to neuromuscular junctions after incubation of normal muscle with serum in 38 of the 40 dogs with AChR titers greater than 0.6 nmol/L. In addi-

TABLE 1. Breeds and Numbers of Dogs With Acquired Idiopathic Megaesophagus and Focal MG (in parentheses)

Akita	1 (0)	Labrador Retriever	16 (4)
Bernese Mountain Dog	1 (1)	Lhasa Apso	1 (0)
Bloodhound	1 (0)	Malamute	1 (0)
Border Collie	2 (1)	Miniature Dachshund	1 (1)
Boston Terrier	2 (0)	Miniature Poodle	4 (2)
Boxer	2 (0)	Miniature Schnauzer	4 (1)
Brittany Spaniel	1 (0)	Mixed Breed	8 (3)
Bull Mastiff	1 (0)	Norwegian Elkhound	1 (0)
Chesapeake Bay Retriever	1 (0)	Old English Sheep Dog	2 (1)
Chihuahua	1 (0)	Pekingese	1 (0)
Chow Chow	1 (1)	Pit Bull	1 (1)
Cocker Spaniel	6 (1)	Rottweiler	1 (0)
Collie	5 (0)	Samoyed	1 (0)
Dachshund	2 (0)	Scottish Terrier	1 (1)
Doberman Pinscher	7 (0)	Shar-Pei	1 (0)
English Bulldog	1 (0)	Shetland Sheepdog	3 (2)
English Pointer	1 (0)	Shih Tzu	3 (1)
German Shepherd Dog	25 (8)	Siberian Husky	1 (0)
Giant Schnauzer	1 (0)	Skye Terrier	1 (0)
Golden Retriever	20 (7)	Springer Spaniel	1 (1)
Great Dane	8 (1)	Standard Poodle	1 (0)
Irish Setter	4 (1)	Welsh Terrier	1 (0)
Irish Wolfhound	2 (1)	Yorkshire Terrier	1 (0)

* Boehringer-Mannheim, W. Germany.

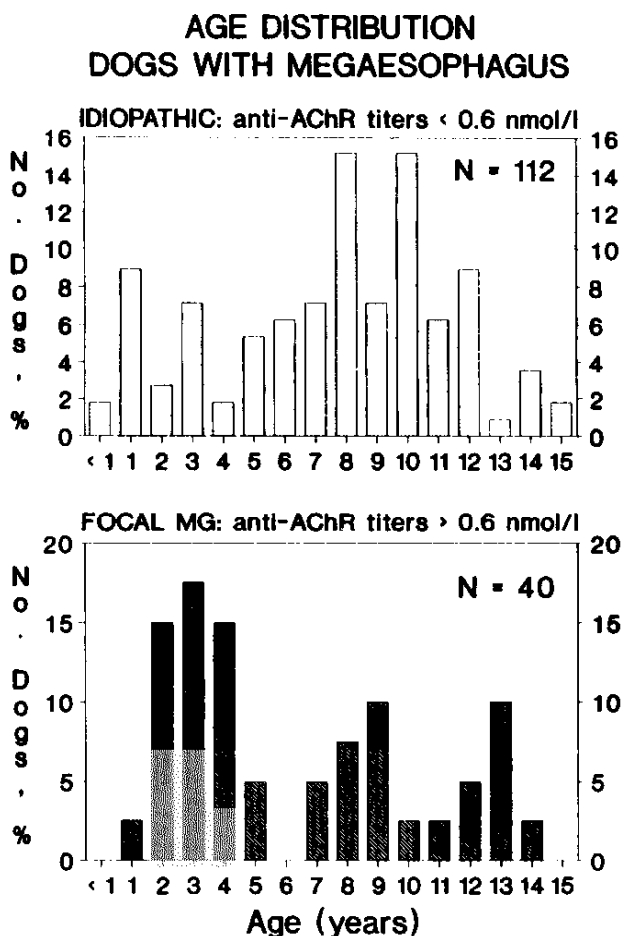


FIG. 1. Dogs with acquired megaesophagus and focal myasthenia gravis have a bimodal age of onset of clinical signs similar to that seen in generalized canine MG. While younger dogs are affected with idiopathic megaesophagus from other etiologies (titers < 0.6 nmol/L), an older age of onset seems to predominate.

tion, the sera of 17 dogs with titers less than 0.6 nmol/L resulted in staining of neuromuscular junctions. In this group of dogs, AChR antibodies were also not detected against the α -Bgt binding site.

Of the dogs with positive AChR antibody titers, the clinical course following diagnosis was available for 35 of 40 cases. Six dogs died of aspiration pneumonia or choking, while 12 were euthanized with no treatment attempted due to the poor prognosis that was given. Of the remaining 17 dogs (48%), 11 improved clinically in association with a decrease in AChR antibody titers, while clinical signs resolved in six dogs with a decrease in antibody titers to less than 0.6 nmol/L. In the six dogs in complete remission, radiographic resolution of the megaesophagus was reported. Clinical remission occurred as early as one month after diagnosis in some cases, while in others, clinical signs and positive titers have persisted for more than one year. Of the 17 treated dogs, seven (41%) were treated only with altered feeding

(elevation of food and water), three (18%) with prednisone alone, six (35%) with pyridostigmine bromide alone, and one (6%) with a combination of pyridostigmine bromide and prednisone.

Discussion

Idiopathic megaesophagus has long been a diagnostic challenge associated with a poor prognosis.⁸⁻¹⁰ In most cases, the underlying disorder is not determined. This study demonstrates that a significant proportion of dogs with esophageal weakness alone, or associated with pharyngeal and facial muscle weakness, have MG. Focal weakness affecting only the extraocular muscles occurs in human MG, and it appears that a similar focal involvement of esophageal skeletal muscles also occurs in canine MG.

The reason(s) for the selective involvement of particular muscle groups is not known. Possible explanations include differences in the safety margins for neuromuscular transmission between the muscle groups¹¹ or antigenic differences in the AChR itself.¹² It has been previously shown that extraocular muscle fibers are classified into two types by the pattern of endplate distribution: fibers with a single endplate (en plaque) and those with multiple endplates (en grappe). Limb muscles are composed of only one type of fiber (single endplate).¹² Sera of patients with ocular MG can distinguish the two forms of endplates indicating antigenic differences of AChRs.¹² The endplate forms in canine esophageal, pharyngeal, and ocular muscles have not been described.

The mean serum titer of 3.1 nmol/L (range, 0.77-30 nmol/L) was lower than our observed value for generalized canine MG ($X = 10$ nmol/L, range, 0.7-93 nmol/L). Similarly, human ocular MG is associated with titers usually less than 2 nmol/L,¹³ while the average titer is about 50 nmol/L for generalized MG.¹

The detection of immune complexes localized at neuromuscular junctions by immunocytochemical methods in sera that did not have detectable AChR antibody titers suggests that there are antibodies directed against junctional antigens other than the AChR. These cases could represent examples of seronegative MG. Seronegative human MG in which 10% to 15% of the patients with generalized MG and 30% to 50% of those with ocular MG have no detectable AChR antibody titers has been reported.^{14,15} Similar results have been observed in canine generalized MG.¹⁶ Explanations presented for this finding include: 1) during the solubilization process involved in AChR preparation, critical determinants on the AChR are lost, 2) a low titer of high affinity antibody could be present with all available antibody bound to AChRs, 3) differences in ability of various AChR preparations to detect low-titer serum, 4) inability of the standard radioimmunoassay to detect

antibodies directed specifically against the α -Bgt binding site, 5) antigenic differences in AChRs as have been shown for extraocular muscles, or 6) autoantibodies directed against endplate determinants other than AChR.

The diagnosis of the focal form of canine MG is best made by demonstrating antibodies against AChRs. A positive titer documents an immune response specifically against muscle endplate AChRs. Immunocytochemical assays should be concurrently performed to detect dogs that may have antibodies against endplate components other than AChR. The Tensilon test, useful for a presumptive diagnosis of generalized canine MG, has not proven useful in the diagnosis of focal MG. In cases involving facial muscle dysfunction, however, an improved palpebral reflex may be observed. If possible, electrodiagnostic testing should be performed on all dogs with the focal form of MG, since mild clinical weakness may not be detected. In two dogs in this study with positive titers in the absence of obvious generalized weakness, the compound muscle action potential decremented upon repetitive nerve stimulation. This indicates that, although weakness was not evident clinically, neuromuscular transmission was not normal in limb muscle. The significance of this finding to focal canine MG will need to be determined by studying a larger group of seropositive dogs. Similar findings have been reported in human ocular MG.¹⁷

Therapy for regurgitating dogs with acquired esophageal dilatation is best accomplished by resolving the underlying disorder if it can be determined, and MG is one such cause that is amenable to therapy. That 48% of the dogs in this study improved or resolved suggests an early diagnosis of MG and initiation of supportive therapy is important to a successful clinical outcome. The esophagus may not respond as favorably to anticholinesterase therapy as limb muscles, and the chance of overdose due to inadequate monitoring of clinical signs is increased.

Unless esophageal distention is severe, resulting in secondary damage, irreversible dilation of the esophagus is unlikely; however, many months of supportive care may be required before it returns to normal size. Hypothyroidism and other autoimmune diseases can occur concurrently with MG, and appropriate therapy should also be instituted for these disorders.

While the etiology of megaesophagus in a large percentage of dogs is still undetermined, it appears that a significant proportion (e.g., 40/152, or 26% in this

study) have acquired MG. Since megaesophagus is a relatively common feature of many generalized neuromuscular diseases, other disorders may cause subclinical weakness in association with megaesophagus. In any case, a favorable clinical course is dependent on early recognition of megaesophagus as the cause of regurgitation and the institution of appropriate symptomatic therapy.

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