An early clinical phenotype of necrotizing meningoencephalitis in the Pug reveals similarities to multiple sclerosis in humans

Rebecca Windsor, DVM, DACVIM\textsuperscript{1}; Samuel Stewart, DVM, DACVECC\textsuperscript{1}; Matt Huentelman, PhD\textsuperscript{2}; Stefan Keller, DVM, PhD\textsuperscript{3}; Chand Khanna, DVM, PhD, DACVIM, DACVP\textsuperscript{1}

\textsuperscript{1}Ethos Discovery, Woburn, MA
\textsuperscript{2}Translational Genomics Research Institute, Neurogenomics Division, Phoenix, AZ
\textsuperscript{3}Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California-Davis, Davis, CA

\textsuperscript{*}Corresponding author: Dr. Windsor (rwindsor@wrah.com)

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ABSTRACT

Necrotizing meningoencephalitis (NME) is a fatal neuroinflammatory disease that previously carried a uniformly grave prognosis. Our recent identification of a novel early form of NME in Pugs suggests that disease onset and progression are likely more insidious than previously recognized and provides new hope that early therapeutic intervention may halt disease progression and ultimately prevent or cure NME. This novel perspective also sheds new light on the clinical similarities to multiple sclerosis (MS) in humans and provides a rationale for cross-species translation. The history of recent scientific discoveries in NME and new parallels between MS and NME will be reviewed.

Keywords: necrotizing meningoencephalitis, multiple sclerosis, neuroinflammatory, autoimmune, immune-mediated

Autoimmune neuroinflammatory diseases are universally common in humans and dogs and serve as a major source of chronic disability and fatality. Multiple sclerosis (MS) is the most common neuroinflammatory disease in humans and shares several similarities with meningoencephalitis of unknown origin (MUO) in dogs, most specifically with the necrotizing meningoencephalitis (NME) subtype. The most notable similarities include genetic, age, race/breed, and sex predisposition for MS in humans and NME in dogs and the high fatality rate for both diseases. There have been several advancements in the past 10 to 15 years toward understanding the epidemiology, risk factors, and clinical progression of NME in the Pug.\textsuperscript{1–7} The classic and historically recognized form of NME (henceforth referred to as “classic NME”) closely mimics fulminant forms of MS in humans (ie, Marburg variant). Dogs with classic NME are typically diagnosed when clinical signs are severe and MRI and pathological changes are advanced; however, the true progression of the disease is unknown. Although NME has been previously associated with rapid progression and guarded prognosis, it may represent the culmination of chronic progressive pathology where early subtle neurological abnormalities are not readily apparent. Our recent identification of a clinical phenotype that we suspect represents an early form of NME in genetically at-risk Pugs (henceforth referred to as “early NME”) suggests that NME may have a longer disease course than originally understood.\textsuperscript{7} We still have much to learn as to whether early NME precedes classic NME or whether there could be a spectrum of NME in dogs with different stages and degrees of aggressiveness, more akin to the prototypic relapsing-remitting form of MS (RRMS) in humans. This review provides a side-by-side comparison of MS in humans and NME in Pugs with a focus on clinical progression, risk factors for disease development, MRI, pathological, and immunological features of disease, and systemic biomarker evaluation. Our therapeutic studies in Pugs with early NME suggest that early immunomodulation may be an important part of a strategy to halt and reverse chronic neuroinflammatory changes. Our ongoing research asks whether a proactive approach to managing NME should include routine screening of young Pugs to identify the early clinical phenotype, genetic risk assessment, systemic biomarker analyses, treatment with immunomodulatory medications including mesenchymal...
stem cells (MSCs), and subsequent therapeutic monitoring using systemic biomarkers and MRI. The parallels between MS in humans and NME in dogs highlight the potential for the Pug as a translational animal model for MS.

Initial Discovery of MS and NME

Reports of humans with MS-like symptoms (reduced vision, weakness, pain) date back to the late 1300s, and the plaque-like lesions associated with MS were first described by a Parisian neurologist in 1868. Despite the increasing knowledge of MS over the following 150 years, early treatment regimens for MS were not published until the late 1980s/early 1990s. Multiple sclerosis currently affects approximately 2.5 million people worldwide and serves as the leading cause of nontraumatic disability in young adults.

Necrotizing meningoencephalitis in Pugs, or Pug dog encephalitis (PDE), was first clinically recognized in the 1960s; however, the first pathological description of NME was not published until the late 1980s. Clinical recognition has until recently been limited to an acute fulminant presentation that shortly precedes death in most dogs (classic NME). Necrotizing meningoencephalitis is included under the umbrella term of MUO, which includes the more common granulomatous meningoencephalomyelitis (GME) and less common necrotizing leukoencephalitis (NLE); however, the unique pathological characteristics and narrow breed predisposition for NME distinguish it from GME.

Genetics of MS and NME

The greatest genetic risk factor for MS is the major histocompatibility complex (MHC) human leukocyte antigen-DR15 haplotype designated as *15:01, which contributes to approximately 60% of the risk in Whites of European descent. MHC genes determine immune repertoire, and the identified risk loci in humans contain many immunologically relevant genes that suggest T-cell alterations likely factor in the pathogenesis of MS. Several other risk-associated genes have been identified that are virtually all immune response genes. Genes coding for inflammatory cytokines and non-MHC genes that determine regulatory and tolerance mechanisms are also altered in humans with MS.

Genetic risk for NME is similar and most closely associated with a haplotype within the dog leukocyte antigen (DLA) MHC class II complex. Additional polymorphisms have been identified within genes coding for immune function within chromosome 15 in the Pug and chromosome 4 in the Maltese. Current estimates suggest that approximately 2/3 of Pugs carry at least 1 DLA at-risk haplotype, and 6% to 18% of Pugs carry the high-risk homozygous DLA haplotype.

Disease stages of MS and NME

Stages of MS

Multiple sclerosis is characterized by progressive, relapsing-remitting, and fulminant forms. Classic NME in dogs closely resembles fulminant MS in humans; however, our recent identification of an early clinical presentation of NME that shares many similarities to the early phases of prototypic MS suggests there is likely a more chronic form of NME that was not previously recognized. We now suspect that these neuroinflammatory diseases may share similar clinical presentations and courses of disease. The study of the more chronic presentation of NME in Pugs is a focus of our ongoing clinical and biomarker-based research.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>MS in Humans</th>
<th>NME in Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic variation</td>
<td>Human leukocyte antigen (HLA)-DR15</td>
<td>Dog leukocyte antigen (DLA) major histocompatibility complex II (MHC II) chromosome 12</td>
</tr>
<tr>
<td>Gender/sex predisposition</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>Age at onset</td>
<td>Young, 20–40 years Younger for fulminant form</td>
<td>Young (&lt; 6 years)</td>
</tr>
<tr>
<td>Race/coat color</td>
<td>Caucasian/White</td>
<td>Fawn</td>
</tr>
<tr>
<td>Triggers of disease onset</td>
<td>Smoking Low vitamin D Previous viral infection Other autoimmune diseases</td>
<td>None yet identified</td>
</tr>
<tr>
<td>Stages of disease</td>
<td>Preclinical MS Radiographically isolated syndrome (RIS)</td>
<td>Preclinical NME</td>
</tr>
<tr>
<td>Early MS</td>
<td>Clinically isolated syndrome (CIS)</td>
<td>Early clinical phenotype</td>
</tr>
<tr>
<td>Prototypic MS</td>
<td>Relapsing-remitting MS (RRMS) Secondary progressive MS (SPMS) Neurodegenerative stage</td>
<td>Phases not yet recognized</td>
</tr>
<tr>
<td>Other MS variants</td>
<td>Primary progressive MS (PPMS) Fulminant MS (ie, Marburg variant) Silent MS</td>
<td>Chronic NME Classic/fulminant NME Variant not yet recognized</td>
</tr>
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</table>
risk factors and disease stages for MS in humans and NME in dogs.

Preclinical MS—The preclinical phases of MS include an initiation and latency phase. The initiation phase is thought to arise between 5 and 20 years of age, and although there are no obvious clinical symptoms, immunological processes including activation of autoreactive lymphocytes and altered lymphocyte physiology are already occurring. In the following 10 to 20 years during the latency phase, inflammatory cells accumulate within the brain and demyelination begins to occur before any apparent symptoms arise. In this preclinical phase, a large number of MRI lesions are already forming. Cognitive performance is lower in people in this preclinical stage of MS compared to age-matched controls, indicating cognitive dysfunction may occur before humans manifest overt symptoms of MS.

Clinical MS—In the early stages of clinical disease, known as clinically isolated syndrome (CIS), humans exhibit their first symptoms of MS, which typically include blurred vision; hyperesthesia, which often manifests as a tingling feeling in the distal limbs; and reduced proprioception often characterized by intermittent tripping. This stage is associated with demyelination within the cortex and white matter and can be triggered by other systemic inflammatory stimuli. As the disease progresses into RRMS, the symptomatic attacks become more frequent with periods of stability in between. Clinical relapses may correlate with new waves of inflammatory cell infiltration across the blood-brain barrier (BBB), recurrent bouts of demyelination and axonal injury, and progressive cortical pathology with variable ability for the nervous system to repair it. Anti-inflammatory therapies are often effective in these early stages.

During the transition to secondary progressive multiple sclerosis, the nervous system is no longer able to repair, anti-inflammatory therapies become increasingly less effective, and people do not fully recover from attacks. In the final stage of the disease, known as the neurodegenerative phase, the neurological decline is steadily progressive resulting in marked physical disability. This correlates with widespread brain pathology, glial activation, axonal degeneration, and meningeal inflammation. No therapies are effective in this stage of MS.

Approximately 10% of the population has benign MS, where the disease causes no substantial clinical impact. Another 10% to 15% of humans present with progressive symptoms without manifesting the initial relapsing-remitting phase, referred to as primary progressive MS. Fulminant MS—Approximately 7% of people present with an acute, rapidly progressive fulminant form of MS, which is unique from other subtypes and includes the Marburg variant. People often develop multifocal/diffuse central nervous system (CNS) signs including seizures, decreased sensation, hyperesthesia/paresthesia, and visual field deficits. These fulminant forms occur in younger people compared to prototypic MS. MRI often shows severe inflammatory lesions with marked contrast enhancement, edema, and in some cases mass effect. Many of the lesions exhibit some degree of T1-weighted hypointensity suggestive of necrosis. Electrodiagnostic testing demonstrates disruption within the visual evoked potential in up to 1/3 of cases and disruption of the somatosensory potential in up to 60% of cases. Brain pathology shows severe axonal injury, necrosis, and dense infiltration of inflammatory cells. Humans with these aggressive forms of MS have a clinical picture that is very similar to classic NME in dogs.

Clinical Manifestations of NME

Classic NME—Necrotizing meningoencephalitis has been historically associated with acute rapidly progressive signs and a clinical picture and MRI features that are nearly identical to the fulminant forms of MS. The most common signs demonstrated at the time of diagnosis include seizures, circling, visual deficits, behavior change, and lethargy. Treatment with corticosteroids and immunosuppressive medications is typically futile given the severity of the brain pathology and irreversible necrosis at the time of diagnosis.

Chronic NME—A small subset of dogs with NME manifests a gradual clinical progression with subtle MRI findings at the time clinical signs occur and survival times of 2 to 3 years instead of weeks to months. It is possible that dogs with chronic NME represent a different variant of the disease.

Early NME—We have recently described a clinical phenotype of NME that we now define as early NME. The majority of Pugs with this phenotype were identified during screening clinics between 6 months and 3 years of age with the exception of 3 dogs that had a history of seizures in the preceding weeks. The neurological abnormalities associated with this early clinical phenotype include multifocal spinal hyperesthesia and paw placement deficits in all dogs, reduced menace response in approximately 90% of dogs, mild lethargy in approximately 75% of dogs, and proprioceptive ataxia in approximately 65% of dogs. Many of these signs mirror the neurological abnormalities noted in humans in the earliest stages of MS (ie, CIS), which include visual deficits, proprioceptive abnormalities, and sensitivity to touch. Reduced cognitive function has also been reported by owners of some dogs with early NME, similar to the cognitive changes noted in some humans with preclinical MS. At this stage it is unknown whether dogs with early NME will ultimately develop a chronic form of NME similar to the prototypic form of MS or progress to develop the necrotic lesions seen with the classic form of NME.

MRI findings in MS and NME

The increasing availability of MRI has greatly improved the ability to diagnose MS in the early stages. The hallmark MRI findings of MS include T2-weighted hyperintense and T1-weighted post-contrast enhancing lesions, commonly found...
adjacent to the lateral ventricles. Brain and spinal cord atrophy are also common due to axonal and myelin loss. The reliability of these findings allows conventional MRI measures to be used to monitor response in clinical trials. MRI lesions begin forming in the preclinical stages of MS, a condition known as radiographically isolated syndrome, and can often exist as small solitary lesions (Figure 1). New MRI lesions continue to form throughout RRMS; however, by secondary progressive MS, few new lesions arise.

Magnetic resonance imaging findings in dogs with classic NME show loss of gray/white matter distinction, parenchymal inflammatory/necrotic lesions, and leptomeningeal contrast enhancement. These changes are strikingly similar to the MRI findings in humans with the fulminant forms of MS (Figure 2). In rare cases of chronic NME, MRI lesions can be more subtle initially and progress to more obvious necrotizing lesions over years. MRI changes are identified in approximately 90% of dogs with early NME, which include meningeal enhancement, focal contrast-enhancing lesions within the cerebral cortical parenchyma, and T2-weighted hyperintense lesions that could be consistent with early necrosis. Parenchymal lesions are most commonly identified in the parietal and occipital lobes, which correlates with previously reported lesion distribution for Pugs with NME. These solitary lesions resemble the early focal lesions seen in humans with radiographically isolated syndrome and CIS (Figure 1).

**Cerebrospinal fluid cytology in MS and NME**

Cerebrospinal fluid cytology is not reliable to diagnose MS in humans; greater emphasis is placed on inflammatory biomarkers including oligoclonal...
bands and κ-free light chain.\textsuperscript{28-30} Approximately 40 to 50% of people with prototypic MS have a CSF pleocytosis (median 10 cells/μL, upper limit approx 40 cells/μL) with lymphocytic/mononuclear cell predominance; a mild protein elevation of up to 60 g/dL is seen in approximately 25% of patients.\textsuperscript{25} Presence of CSF inflammation and overall WBC count trends lower in humans with CIS.\textsuperscript{24} Cytology is often normal in humans with fulminant MS, but when abnormal typically shows mild mononuclear or lymphocytic inflammation and increased protein.\textsuperscript{21}

Cerebrospinal fluid changes seen with classic NME include lymphocytic pleocytosis and increased protein in the majority of dogs and normal CSF in 10 to 20% of dogs.\textsuperscript{1,10,31,35} CSF cytology is abnormal in approximately 50% dogs with early NME with changes including lymphocytic pleocytosis, protein elevation, and presence of a basophil in 1 dog (cell type associated with neuroinflammatory diseases in humans).\textsuperscript{7}

### Pathological findings of MS and NME

Multiple sclerosis is characterized by an initial inflammatory phase that may be triggered by damage within CNS tissue; damaged neural tissue may then perpetuate secondary inflammation.\textsuperscript{13} Inflammatory white matter lesions can arise anywhere but have a predilection for the periventricular white matter.\textsuperscript{18} Leptomeningeal and gray matter involvement are also initial targets in early MS.\textsuperscript{21} The inflammatory process is believed to destroy the oligodendrocyte cell body or myelin sheath, causing demyelination, which is the hallmark sign of MS.\textsuperscript{13,18} Axonal damage and gliosis are likely to follow in the later stages.\textsuperscript{13,18} Location of gray matter demyelination and atrophy and diffuse white matter injury contribute to the clinical picture associated with MS, and gray matter atrophy is associated with greater long-term disability.\textsuperscript{18} Although remyelination occurs in most people indicating the potential for the nervous system to repair, natural mechanisms of repair and regeneration are limited.\textsuperscript{13,18} Estimates of 10% to 25% of people diagnosed with MS on histopathology are clinically silent.\textsuperscript{9,33} and anatomical distribution of MS plaques (focal areas of myelin loss) shows a predilection for the periventricular white matter compared to a more disseminated distribution in patients with clinical MS.\textsuperscript{13} Pathological findings in the fulminant forms of MS are similar to NME with prominent tissue necrosis secondary to inflammatory demyelination and macrophage infiltration.\textsuperscript{10,21}

Brain histopathology in dogs with classic NME shows predominant lymphocytic inflammation, perivascular cuffs, and necrosis within the cerebral cortex.\textsuperscript{10,31,15,35} Three pathological phases of NME have been described, which include an acute phase characterized by mild inflammation, a subacute phase with more severe inflammatory change and moderate malacia, and chronic form with extensive malacia.\textsuperscript{36} At this time, there is no pathological assessment of dogs with early NME, limiting the ability to compare Pugs with early NME to those with classic NME and to humans with MS. All dogs diagnosed with early NME are still less than 5 years of age and currently responding to therapeutic intervention; pathological assessment will be performed at the time of death.

### Immunology of MS and NME

The pathogenesis and immunological dysfunction in humans with MS is complex and not completely understood but likely involves some contribution by CD4+ T cells, cytotoxic CD8+ T cells, B cells, and macrophages.\textsuperscript{12} A predominant T-cell-mediated response is suspected to be characterized by myelin-specific CD4+ T helper 1 (Th1) and Th17 cells and CD8+ myelin autoreactive T cells triggering the neuropathology.\textsuperscript{12,13} B cells are also thought to play a central role in MS and may enhance the local CNS response in the progressive stage of MS.\textsuperscript{12}

Immunological MS research is largely focused on understanding the origin of the inflammatory process, which is now expected to occur within the periphery with subsequent penetration into the CNS to stimulate local inflammation and demyelination.\textsuperscript{37} T cells within the periphery are likely activated by a viral antigen or superantigen that shows some mimicry of a CNS antigen.\textsuperscript{13} Activated Th1 and Th17 cells capable of producing several proinflammatory cytokines are able to cross the permeable BBB. Release of cytokines and chemokines recruit other immune cells including B cells and monocytes/macrophages to follow into the CNS.\textsuperscript{13} Antigen that is likely derived from myelin is presented by CNS resident or immigrant antigen-presenting cells including microglia, dendritic cells, and astrocytes.\textsuperscript{13} Once presented with this antigen, autoreactive T cells produce inflammatory cytokines, which stimulate neighboring immune or neural cells to attract other inflammatory cells into the CNS.\textsuperscript{13} Resolution of the inflammation comes when and if anti-inflammatory cytokines such as IL-10 and regulatory T cells and natural killer cells come to aid in the repair of the damaged tissue.\textsuperscript{13}

Prototypical MS shows a predominance of T cells histopathologically, with up to 10 times the number of T cells than B cells.\textsuperscript{38} T-cell inflammation is pronounced within active parenchymal lesions and the meninges of humans with MS.\textsuperscript{38,39} CD4+ T cells often predominate in acute lesions whereas CD8+ T cells are observed more frequently in chronic lesions.\textsuperscript{38,39} CD3+ T cells are represented in similar densities in some MS patients.\textsuperscript{38,39} The variety of T-cell subtypes highlights the probable heterogeneity in T-cell responses.\textsuperscript{39} Immunohistochemical analysis with full-mant MS can include infiltration of T and B cells.\textsuperscript{21}

Immunohistochemical analysis of the brain tissue in dogs with NME supports an immune-mediated pathology with some similarities to MS. CD3+ T cells adhered to glial fibrillary acidic protein-positive astrocytes have been identified in the peripheral malacic regions in dogs with NME but also in dogs with GME.\textsuperscript{31,36,40} CD20+ B cells have also been identified in dogs with NME, NLE, and GME.\textsuperscript{31,36} IgG-positive cytoplasm and astrocytic processes have
been identified in dogs with NME that were in greater abundance than NLE and not present in GME. More extensive immunohistochemical analysis of T cell populations is required to better understand the immunological processes of NME in the dog and similarities to MS in humans.

Systemic biomarker analysis in MS and NME

Blood and CSF inflammatory biomarkers are being heavily explored in human and canine neuroinflammatory disorders to predict disease development and progression and monitor response to therapy. Serum biomarkers have the advantage of being easier and less invasive to obtain, whereas CSF biomarkers may more accurately reflect local inflammatory and degenerative processes in the brain. There is currently no ideal systemic biomarker for NME, and normal, early clinical, and more severely clinically affected Pugs need to be adequately compared to best identify a valuable systemic biomarker. Biomarker data specific to NME are generally very limited as many dogs do not have histopathological confirmation of NME at the time of biomarker analysis. Serum and CSF biomarker analysis in dogs with MUO is more readily available given the specifications for premortem diagnosis; however, the immunological processes of this broader category of neuroinflammatory disease may not accurately reflect those of dogs within the NME subcategory. A comprehensive biomarker review for MUO and NME has been recently published and is beyond the intended scope of this publication. A noncomprehensive list of biomarkers of MS is summarized (Table 2) with comparative data for dogs with NME or, when unavailable for NME specifically, for dogs with MUO. Detailed information regarding systemic biomarker evaluation is available elsewhere (Supplementary Material S1).

Table 2—Systemic biomarkers for multiple sclerosis (MS) in humans and necrotizing meningoencephalitis (NME) or meningoencephalitis of unknown origin (MUO) in dogs.

<table>
<thead>
<tr>
<th>Biomarker Type</th>
<th>MS</th>
<th>NME</th>
</tr>
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<tbody>
<tr>
<td>CSF cytology</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Mononuclear/lymphocytic</td>
<td>Lymphocytic</td>
</tr>
<tr>
<td></td>
<td>Increased protein</td>
<td>Increased protein</td>
</tr>
<tr>
<td>Oligoclonal bands (OCBs)</td>
<td>Frequently detected</td>
<td>Identified in MUO⁴²</td>
</tr>
<tr>
<td>Glial fibrillary acid protein (GFAP)</td>
<td>Elevated, can correlate with disability</td>
<td>Elevated in NME and healthy Pugs⁴³-⁴⁵</td>
</tr>
<tr>
<td>Serum cytokines (CK)</td>
<td>Elevated proinflammatory CK</td>
<td>Elevated IL-10 in normal at-risk Pugs¹⁵</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Elevated IFN-γ in NME⁴⁶</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Elevated IL-17 in MUO⁴⁷</td>
</tr>
<tr>
<td>CSF cytokines</td>
<td>Alterations in prototypic and fulminant forms</td>
<td>Not yet evaluated</td>
</tr>
<tr>
<td>Glutamate</td>
<td>Elevations with relapse and progression</td>
<td>Elevations in NME⁴⁸</td>
</tr>
<tr>
<td>Lactate</td>
<td>Used to monitor disease progression and response to therapy</td>
<td>Elevations in blood⁴⁹ and CSF⁵⁰ of dogs with MUO</td>
</tr>
<tr>
<td>Kappa free light chain (κ-FLC)</td>
<td>Elevations with increasing disability</td>
<td>Not yet evaluated</td>
</tr>
<tr>
<td>Neurofilament light (NFL) chain</td>
<td>Good correlation with early MS activity</td>
<td>Elevated in MUO⁵¹</td>
</tr>
<tr>
<td>T-cell receptor (TCR) repertoire</td>
<td>Greater TCRα and TCRβ diversity Clusters in humans with HLA-DRB1 TCRβ changes with therapy</td>
<td>Under evaluation</td>
</tr>
<tr>
<td>Proteomics</td>
<td>GFAP⁵²</td>
<td>Not yet evaluated</td>
</tr>
<tr>
<td></td>
<td>CHI3L proteins⁵²</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SERPINA3⁵²</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C4 protein⁵²</td>
<td></td>
</tr>
<tr>
<td>Fecal microbiome</td>
<td>Elevated gut permeability</td>
<td>Prevetellaceae less abundant in MUO⁵³</td>
</tr>
<tr>
<td></td>
<td>Decreased bacteria with immunomodulatory properties</td>
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and teriflunomide, a metabolite of leflunomide. Failure rates in treating fulminant MS are very high, with survival rates of < 40% of patients and favorable outcomes in < 20% of patients. Some success has been achieved with the use of cyclophosphamide and mitoxantrone in humans with the Marburg variant of MS.

Necrotizing meningoencephalitis classically shows a very poor response to immunosuppression, largely because it is impossible to reverse/repair the necrosis by the time clinical signs manifest in the classic form of NME. Median survival time for classic NME is 6 to 7 months. This is similar to treatment outcomes in humans with fulminant MS and those in the secondary progressive phase of MS where the ability for neural tissue to repair is limited. One Pug with chronic NME survived 2 years after onset of clinical signs. At this stage, it is unknown whether dogs with early NME will ultimately progress to have a chronic form of NME or develop fulminant NME with advanced necrosis. Ethical considerations may limit our ability to obtain this data; to date, we have enrolled all Pugs with early NME into therapeutic trials and reversed neurological signs in all dogs.

Hematopoietic stem cell transplantation has been employed in humans with MS, aiming to reset an immune system prone to autoimmune responses. Intrathecal and intravenous MSCs have been used successfully in dogs with MUO that did not respond to corticosteroid therapy. A prospective clinical trial evaluating the use of MSCs in Pugs with early NME is ongoing at our institution.

Future management for NME should be directed at identifying and treating dogs with early NME, modeling after MS treatment strategies where the aim is to identify humans in preclinical or early stages of MS when medications are more likely to be effective. Our current recommendation is to screen all Pugs starting at 6 months of age (or younger if clinically abnormal) for neurological abnormalities that may suggest early NME, most reliably asymmetrical menace and paw placement deficits and spinal hyperesthesia. Although the majority of Pugs with early NME are genetically at risk, Pug/Chihuahua crosses may not demonstrate the at-risk haplotype identified in purebred Pugs. Genome-wide association studies of Pug/Chihuahua crosses with early NME are currently in progress at our institution. Pugs with progressive neurological deficits consistent with early NME that lack structural abnormalities on MRI to explain deficits and have MRI/CSF findings supporting early NME should be considered for therapeutic intervention.

**NME as a natural animal model for MS**

Although not identical, the similarities in the neuroinflammatory infiltrates and clinical features of NME in dogs and MS in humans suggest a potential for NME to serve as a translational model for early intervention or prevention strategies for MS. Experimental autoimmune encephalomyelitis (EAE) in rodents is currently the primary animal model for MS; however, several differences between the 2 diseases including lack of genetic and environmental factors that influence disease progression in EAE, pathological distribution as predominantly spinal cord in EAE versus brain in MS, and differences in cytokine patterns and drug pharmacodynamic and pharmacokinetic profiles between the species suggest this rodent model may not be ideal to allow translational research to occur. The primary advantages of NME as a model for MS include that it is a naturally occurring neuroinflammatory disease with genetic and environmental stimuli, the comparable species size, ease of MRI acquisition in dogs, potential for comparable biomarker assessment between dogs that may extrapolate to humans, and the opportunity to conduct disease prevention research in a disease that shares the complexity of its human equivalent. The ability to identify early NME in young dogs and typical development of classic NME by 6 years of age enables this research to be conducted in a relatively short time frame. The specific focus of our ongoing biomarker-driven prevention and early intervention studies includes the use of immunomodulatory MSCs in dogs with early NME to halt disease progression and prevent the development of fulminant disease.

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Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org