Course 3
The Association of Polyneuritis and EPM
#7866 **1 Hour CE**

HORSES WITH SUSPECTED EPM HAVE SERUM ANTIBODIES AGAINST REACTIVE AMINO ACIDS OF EQUINE MYELIN PROTEIN

Intern J Appl Res Vet Med 2015 Ellison et al. p 164-170

Intern J Appl Res Vet Med 2015 Ellison et al. p 175-181

EQUINE PROTOZOAL MYELOENCEPHALITIS

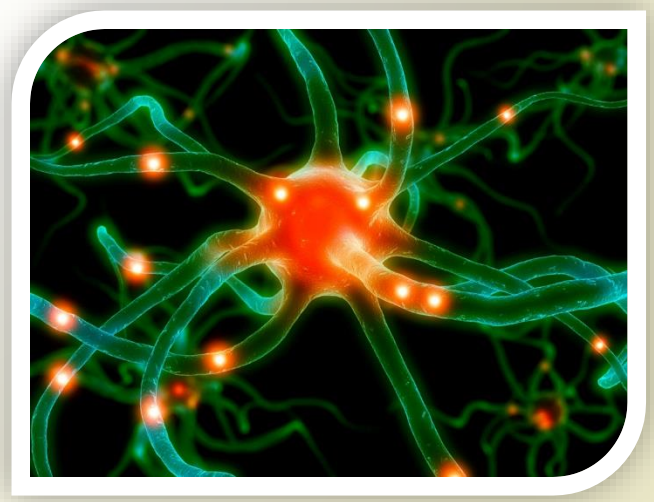
- **EPM** is the most commonly diagnosed neurodegenerative disease in horses
- Inflammation is present in all histologically diagnosed cases of EPM while parasites are rarely found
- Disease is likely due to infectious and non-infectious inflammatory mechanisms

DIAGNOSTIC TESTS FOR EPM-MIMICING DISEASE

- Pre and post disease for viral encephalomyelitis
- Lyme testing (Cornell)
- *S. neurona* antibodies to SAG 1, 5, 6 or IFAT
- Limits of the tests are:
  - There are no pathopneumonic tests
  - These diseases may use a common pathway for inflammation
  - No tests for inflammation are specific to pathogen
- Response to therapy for non-infectious causes EMND and EMD

NEUROINFLAMMATORY DISEASES IN HORSES

- Horses are bright, alert, non-febrile with a normal CBC and chemistry
- Ddx: *S. neurona*, EHV, Neuroborreliosis, polyneuritis equi (**PE**)
- All have similar clinical presentations
- *S. neurona*, EHV, and Lyme require r/o alternate diagnoses
- Histopathology and serum ELISA diagnose PE



POLYNEURITIS EQUI HAS A POOR PROGNOSIS

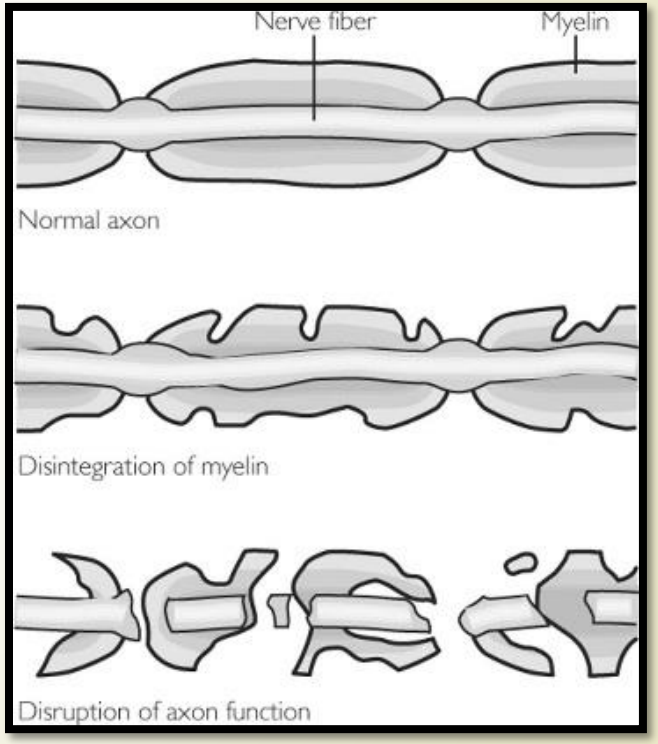
- Cauda equina first described in Germany in 1897
- Found in Europe and the Americas since then
- Progressive, chronic, relapsing/remitting disease affecting the sacral and coccygeal nerves leading to paralysis of the tail, rectum and bladder
- Loss of bowel and bladder function lead to euthanasia

EARLY POLYNEURITIS EQUI

- 1981 - First report that horses with PE have circulating antibodies to myelin P2 protein
- Histopathology from nerve fibers is definitive
- *Cranial nerve deficits and behavior changes early*
- Hyperesthesia followed by hypoesthesia
- Muscle fasciculation!
- Proprioceptive deficits, ataxia

POLYNEURITIS EQUI IS A *PERIPHERAL* DEMYELINATING DISEASE

- Similar to Guillian-Barre syndrome and experimental autoimmune neuritis, **EAN**
- Shown to be unassociated with EHV
- Equine myelin P2 (MP_2) protein or a partial amino acid sequence from EMP2 induces inflammatory demyelinating disease in several species
  - Experimental autoimmune neuritis

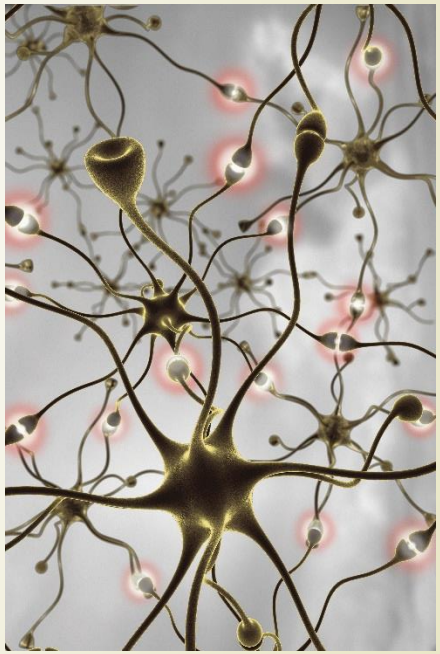


THE HISTORY OF MP2 STUDIES

- Mad Cow Disease in Britain 1986 spurred researchers to find a substitute for bovine neural tissues
- Myelin P2 is conserved across species
- Horses have an unusually large amount of MP2 in the CNS tissues
- Central and peripheral MP2 is identical in amino acid sequence and protein structure

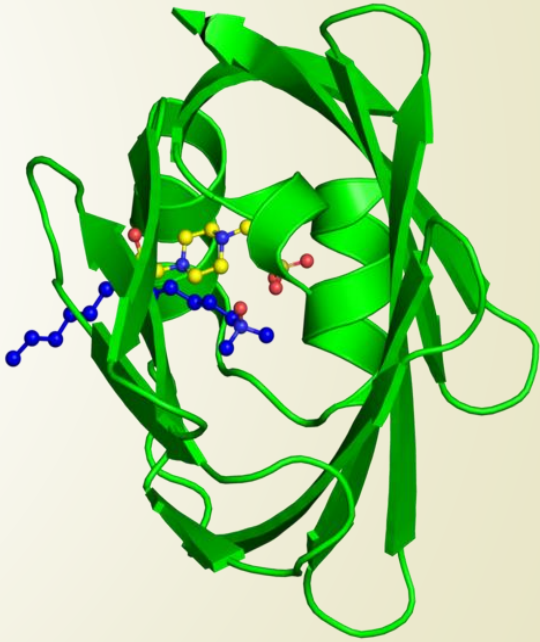
ALMOST CO-INCIDENTIALLY (1981)

- MP2 ELISA detected circulating antibodies to MP2 in neuritis of the cauda equina
- Cauda Equina is more appropriately called polyneuritis equi
- Researchers showed that MP2 challenge causes:
  - Dose dependent peripheral neuritis
  - Individual variation in auto-antibodies
  - Could not predict severity or outcome with ELISA



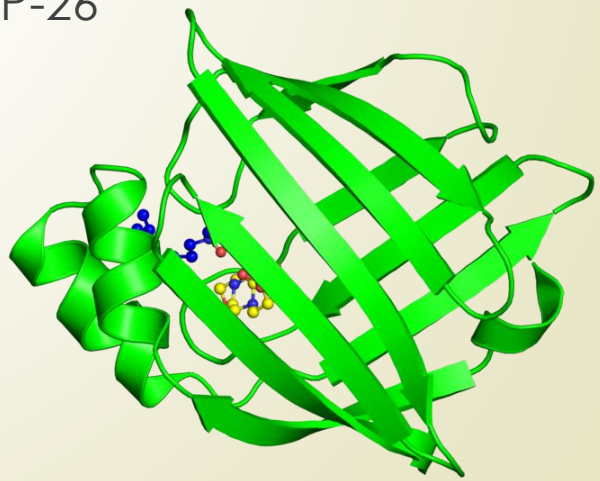
26 aa (SP-26) COMPOSE THE REACTIVE SITE OF MP2

- MP2 or SP-26 induces experimental autoimmune neuritis in rats
- Lymph node cells from SP-26 immunized rats generated reactive T cells specific for SP-26
- SP-26 contains an important T cell epitope for induction of EAN
- SP-26 reactive T cells adoptively transfer EAN to naïve rats

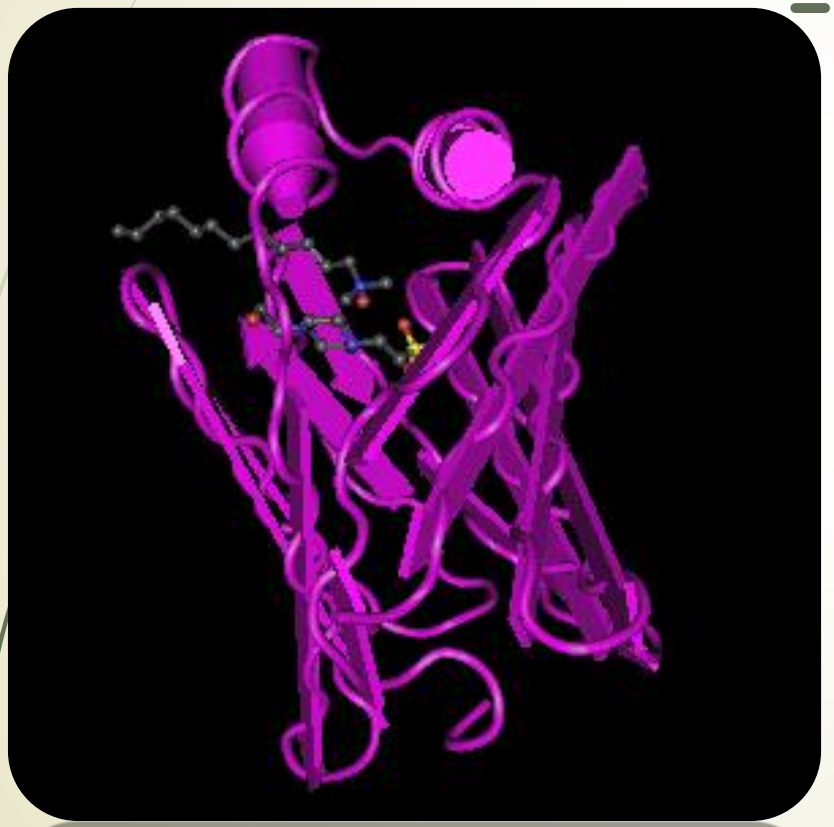


REACTIVE SITE RESIDES WITHIN THE aa 53-78 RESIDUES

- Induced response in rats to whole protein, MP2, was less than induce response to SP-26
- Active T cells lose activity with continued challenge to MP2 **BUT**…
- …Active T cells do not lose reactivity to SP-26
- aa 61-72 does not induce clinical or pathological disease



MPP HAS A REACTIVE SITE AT aa 57-78 but not aa 61-72



GFKNTEISFKLGGEFEETTADN
57 78

Amino acids 57-78 of myelin protein represent MPP and have homology with S. neurona surface antigens

Epitope	Amino acid sequence
SnSAG4	NTEISFKLG
SnSAG6	EFEETTAD
SnSAG5	SFKLG
SnSAG1	FEET
Equine IL6	TTAD

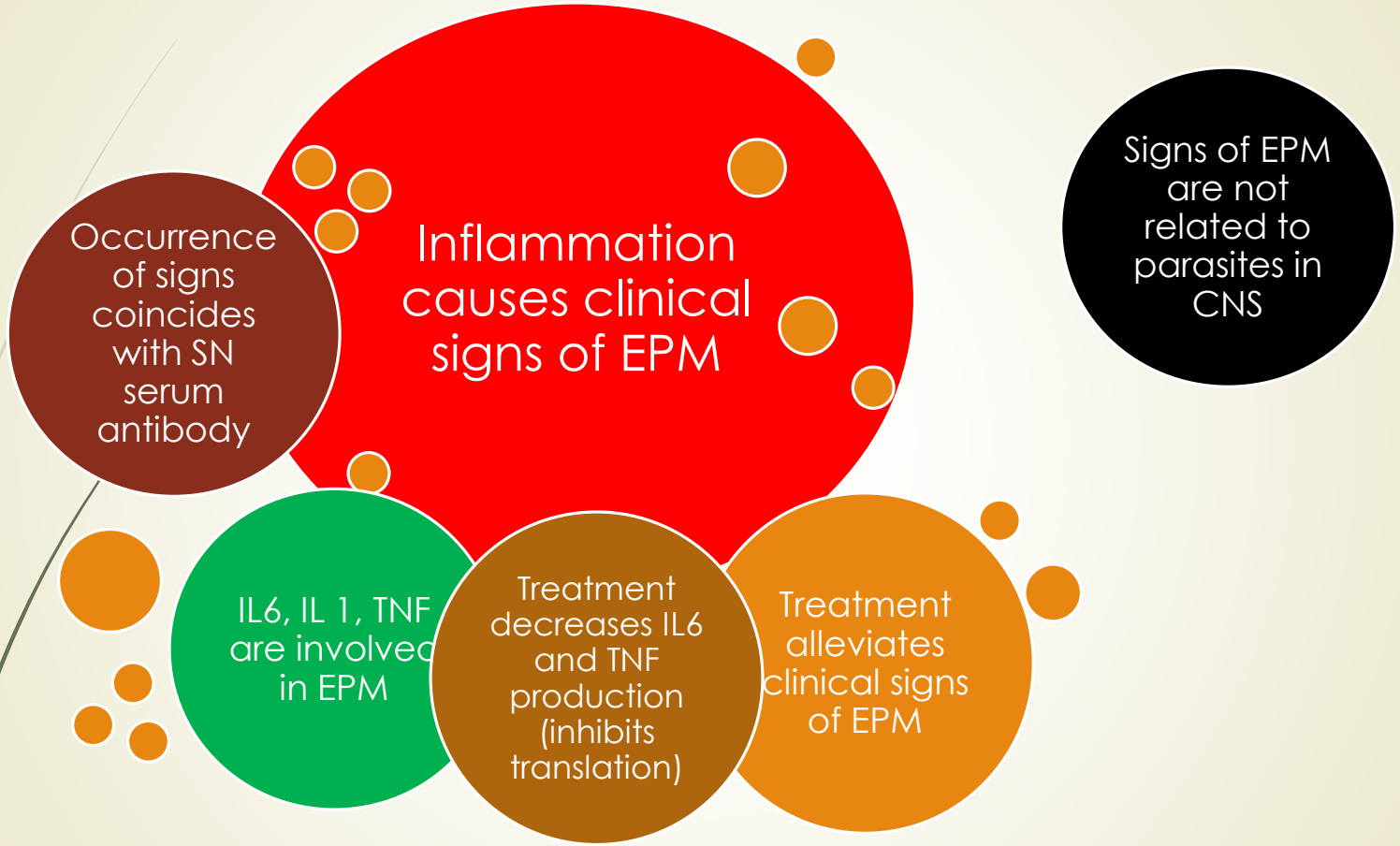
IS IT POSSIBLE THAT POLYNEURITIS EQUI AND EPM SHARE A MECHANISM OF DISEASE VIA IL6?

Circumstantial evidence for an association includes demonstrating blocking IL 6 and alleviating signs in the horse. It is important to demonstrate molecular associations for disease and response to therapy.

ONE CASE

- One neurologically abnormal 12 YO warm blood gelding with serum antibodies against *S. neurona* SAG 1, 5, 6 also had serum antibodies against MPP (titer 64) before treatment.
- The horse was diagnosed with peripheral polymyositis by histopathology (Oregon State University Veterinary Diagnostic Laboratory). Muscle sarcocysts were confirmed by histopathology.
- The horse was clinically normal after treatment to inhibit the expression of IL6. Post-treatment serum antibodies against MPP decreased.

INFLAMMATION MAY BE MEDIATED THROUGH IL6 CYTOKINE PATHWAYS



HOW DOES *S. NEURONA* INCREASE IL6, IL 1, TNF?

- IL 6 binds IL6r to activate JAK/STAT via gp 130 signaling pathway
  - Trans signaling is via soluble IL6-IL6r
  - sIL6r increased in disease
  - Soluble gp130 is antagonistic to IL6-IL6r trans signaling- BUFFERING!
- Homology
  - SnSAG 1 and SnSAG 5 have some homology with IL6
  - SnSAG 3 has homology gp 130
  - SnSAG 4, 6, 5, 1 and IL6 share homology with MPP

- If SAG 1 or SAG 5 bind sIL6r could bind gp 130 for trans signaling, ab to SAG 3 will bind gp 130 increasing trans signaling
- Levamisole can decrease IL6 @ transcription

THE ROLE OF C-REACTIVE PROTEIN IN DISEASE

Generally accepted role of IL6 and CRP in inflammatory disease

- Plasma levels correlate with severity of inflammatory diseases
- Regulated by IL 6
  - IL 6 and serotonin are inversely related
  - IL 6 binds CRP receptors on neutrophils decreasing response
    - Decrease binding to endothelial cells
    - Abates neutrophil recruitment in inflammation
    - Antibody that binds IL6 will decrease CRP
  - anti-IL6 decreases IL6 and can result in a decrease in CRP

IL 6 HOMEOSTASIS

- There are buffers in the serum to maintain homeostasis
  - In steady state sIL-6R and gp130 are 1000x higher than IL 6
  - IL 6 > sIL-6R and gp130 = IL6 acting systemically
    - Only then activates CRP via classic pathway
  - Buffering system makes IL 6 difficult to measure in plasma
  - CRP is easily measured and related to IL6 bioactivity

GENE SIMILARITIES BETWEEN HOST CYTOKINE IL 6, EQUINE MYELIN PROTEIN 2, AND *S. NEURONA* MAY ELICIT COMPLEX IN VIVO INTERACTIONS

- Protozoal homolog's of equine IL6 were found by NCBI blast analysis of Equine IL6 against *S. neurona* data bank with only 3 hits:
  - SnSAG 1 has **44%** sequence similarity with equine IL 6
  - SnSAG 5 has **35%** sequence similarity with equine IL 6
  - Sn enolase (protein kinase) has **28%** similarity with equine IL 6
- Interestingly, SnSAG 4 and SnSAG 6 have no homology with IL 6 while the SAG 4 expressing *falcatula* strain shows 33% homology with IL 6.
  - horses make antibody to SAG 6 indicating infection with SnSAG 6 strains or MPP?
  - Antibody against SnSAG 1 or 6 may influence IL 6 during disease via MPP
- Protozoal homolog of equine gp130 is SAG 3 (25% homology)?

NEUROLOGICALLY ABNORMAL HORSES WERE TESTED FOR SAG 1,5,6 MPP, $MP_{2,}$ ANTIBODY AND SERUM CRP CONCENTRATION BY ELISA*

- Our goal was to determine if horses with EPM have circulating antibodies to a neuritogenic peptide of equine myelin protein and if levamisole HCl is a rational approach to therapy.
- The gait score was evaluated before and after decoquinate/levamisole HCl treatment.
- The serum concentration of C-reactive protein was evaluated pre and post-treatment.
- Ten clinically normal horses were seronegative for MPP ELISA.

REVIEW

Epitope	Amino acid sequence
SnSAG4	60-68
SnSAG6	69-77
SnSAG5	64-68
SnSAG1	71-74
Equine IL6	74-77

- At least one T cell epitope for the induction of EAN resides within the 53-78 residues of myelin P2 protein
- but not within residues 61-72
- An amino acid peptide 61-72 of myelin P2 protein did not induce histological or clinical EAN in rats
- SnSAG 6, 1, and equine IL6 are identical to amino acids 69-77, 71-74, and 74-77, respectively
- We implicate IL6 as a reactive T cell epitope in horses with autoimmune polyneuritis equi by sequence data and effect on CRP.
- SnSAG 4 is identical to amino acids of MPP at residues 60-68, homologous within the non-disease inducing 61-72 residues of myelin P2 protein

DEVELOPING THE TOOL's

- Rabbit anti-MP_2
  - Binds MPP, Mp_2, rSAG 1, rSAG 6 but not rSAG 5
- Horse sera 11482
  - Binds MPP, MP_2, rSAG 1, 5, 6
- Horse sera 11482 can block binding of rabbit anti-MP_2
- Sera from horses with presumptive diagnosis of EPM and neurologically normal horses
- CRP capture ELISA

DEVELOP ELISA'S TO MEASURE MPP AND MP_2

- The MPP ELISA measures serum antibodies to a neuritogenic peptide of equine myelin protein
- The MP_2 ELISA measures serum antibodies to whole equine myelin protein
- The CRP ELISA measures the concentration of acute phase c-reactive protein in serum

WHAT IT MEANS IS THAT THERE MAY BE A LINK BETWEEN EPM AND POLYNEURITIS

- Antibody against MPP indicates the horse has a peripheral neuropathy.
- Antibody against MP_2 indicates the horse has anti-myelin protein antibodies
- We associated peripheral neuropathy with EPM based on proteomics, structure and function, of equine myelin protein and surface antigens of *S. neurona*.
- We associated *S. neurona* SAG's to IL 6 based on amino acid sequence

78% OF ATAXIC HORSES WITH A PRESUMPTIVE DIAGNOSIS OF EPM HAD MPP ANTIBODIES

- Presumptive diagnosis of EPM was based on the presence of antibodies to SAG 1, 5, or 6
- Most horses were MPP seropositive
- A possible mechanism for the neuroinflammatory component of EPM is via IL 6
- The majority of these horses had an elevated CRP

DETERMINING THE APPEARANCE OF MP_2 AND MPP MAY BE USEFUL TO STAGE THE DISEASE PROCESS

- It is possible that treating inflammation in horses with chronic relapsing disease will improve the quality of life.
- A significant number of the horses report insulin resistance and are not amenable to steroid therapy.
- Understanding the relationship between host/parasite and the role in inflammation using proteomics is an most important step in understanding and treating neuromuscular disease.

Quiz

1. Did we convince you there is an association between inflammatory pathways and disease in EPM? Yes No

2. Do you think that IL 6 may mediate inflammation in both polyneuritis and EPM? Yes No

3. If IL 6 production drops (and decreases) is the serum concentration of CRP expected to drop? Yes No

4. Is the most important criteria for the treatment and management of EPM the clinical exam? Yes No