

Measurement and clinical significance of antibodies in neurological diseases

Angela Vincent, Jackie Palace, Stefanie Robb, Linda Clover, John McConville, Natasha Lawrence, John Newsom-Davis, Wei Zhang, Nick Willcox

**Neurosciences Group
and
Department of Clinical Neurology**

***Funded by
Muscular Dystrophy Campaign/Myasthenia Gravis Association
(MDC/MGA), Action Research,
Association Francaise contre les Myopathies (AFM)
The Wellcome Trust, Medical Research Council***

Types of immunoassays

Radioimmunoprecipitation of ^{125}I -neurotoxin-channel
Eg. AChR, VGCC, VGKC

Radioimmunoprecipitation of ^{125}I -antigen
Eg. GAD, MuSK, cytokines

ELISA assays
Eg gangliosides, MuSK

Immunohistochemistry and western blotting
Eg. paraneoplastic, unknown antigens

FACS analysis of Ig binding to cells
Eg. unknown antigens, MuSK

Functional studies - neutralising antibodies
EG. INF beta

Myasthenia gravis

A paradigm for autoimmune disorders

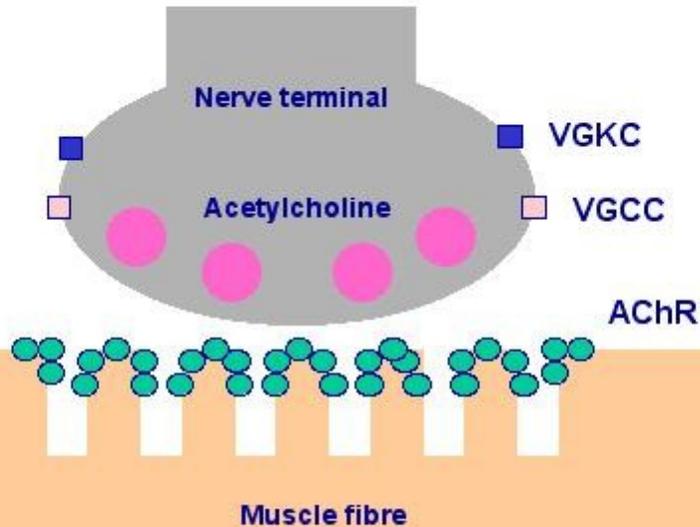
Antibody to extracellular surface of membrane protein

Patients respond to plasma exchange and immunosuppression

IgG injected into mice causes symptoms or signs of disease



Antibodies to ion channels at the neuromuscular junction are measured by radioimmunoassays



- **AChR antibodies in myasthenia gravis**
- **VGCC antibodies in Lambert Eaton myasthenic syndrome**
- **VGKC antibodies in neuromyotonia**

Measuring antibodies by radioimmunoassay

Use tissue or cell extracts of ion channels

Neurotoxins specific for ion channel

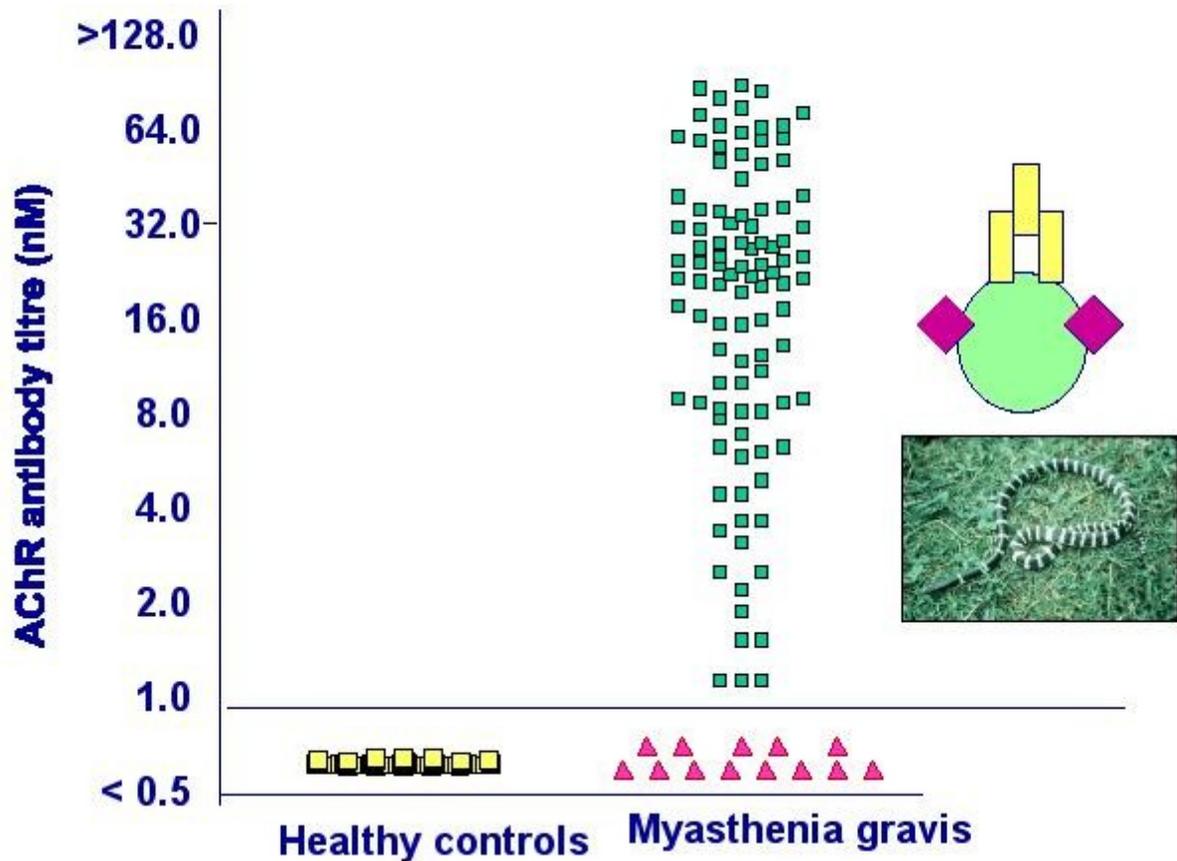
^{125}I -neurotoxin



Measure immunoprecipitation of ^{125}I -neurotoxin-channel complex by patient's serum

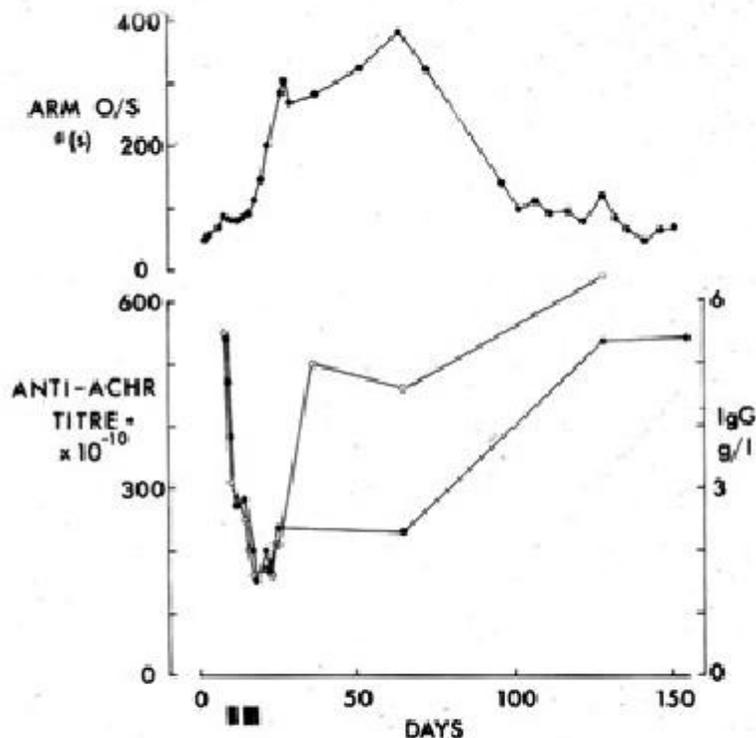
Test is highly specific and quantifiable

AChR antibody levels in MG

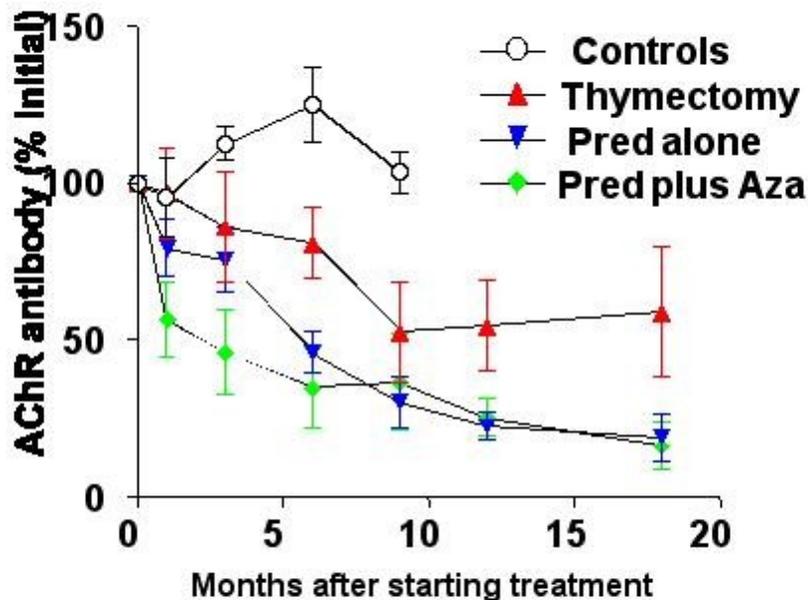


Patients with
myasthenia gravis get
better when their
plasma is exchanged

Newsom-Davis et al
1978



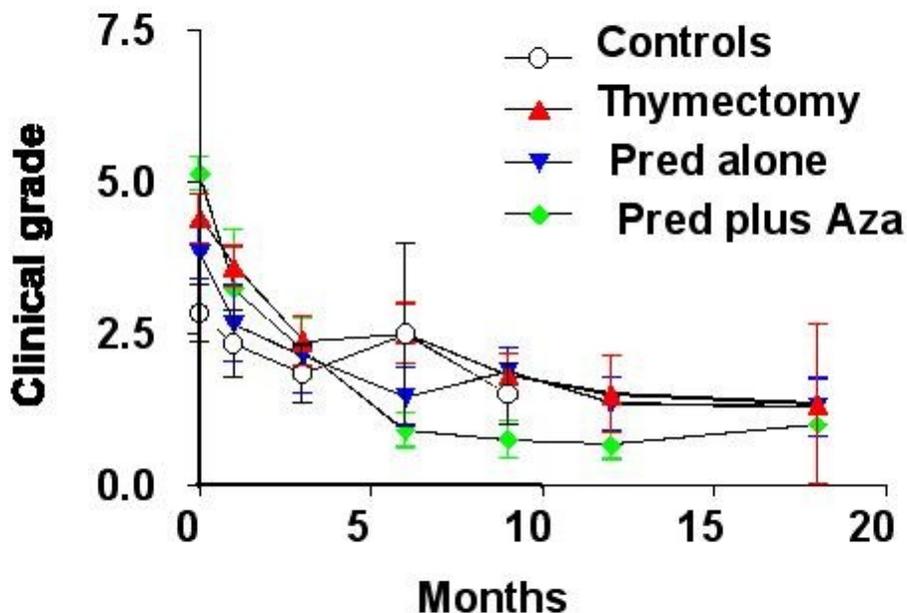
AChR antibodies after different treatments for MG



Do the antibody levels correlate with clinical grade?

Robb et al unpublished data

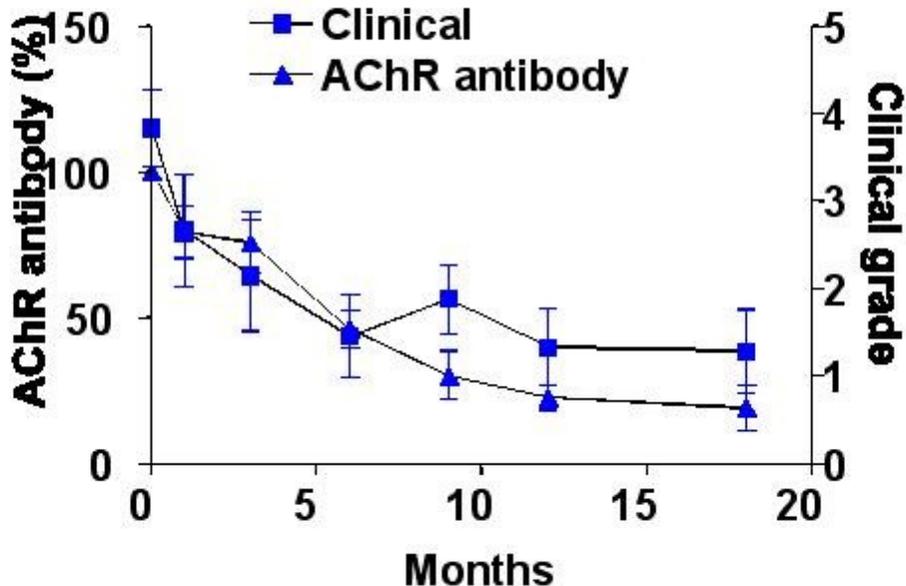
Clinical grades after different treatments for MG



Robb et al unpublished data

AChR antibody and clinical grades after different treatments for MG.

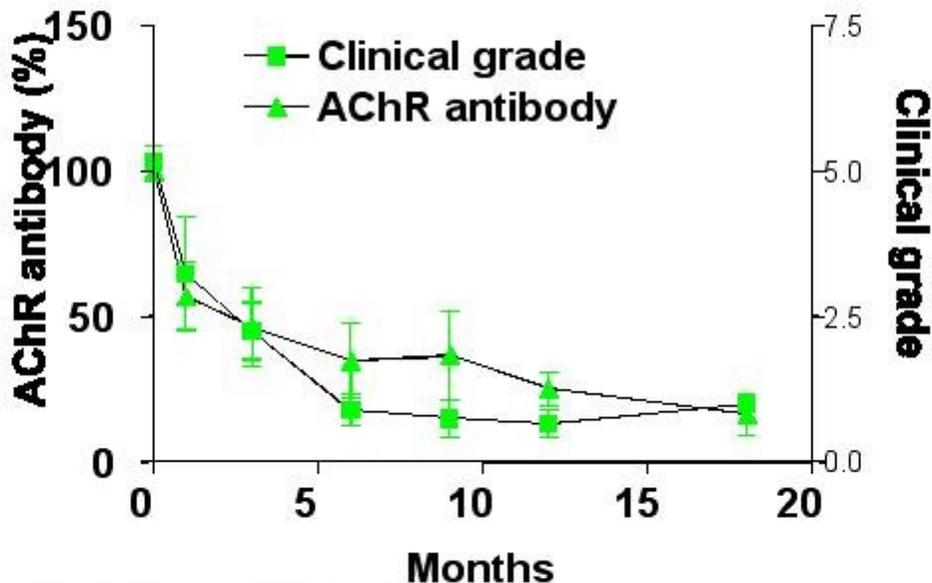
Prednisolone alone



Robb et al unpublished data

AChR antibody and clinical grades after different treatments for MG.

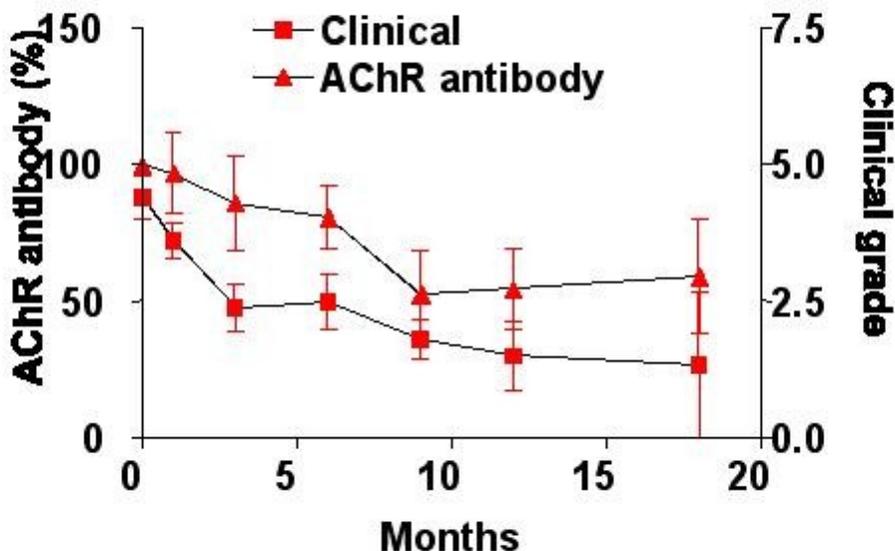
Prednisolone and azathioprine



Robb et al unpublished data

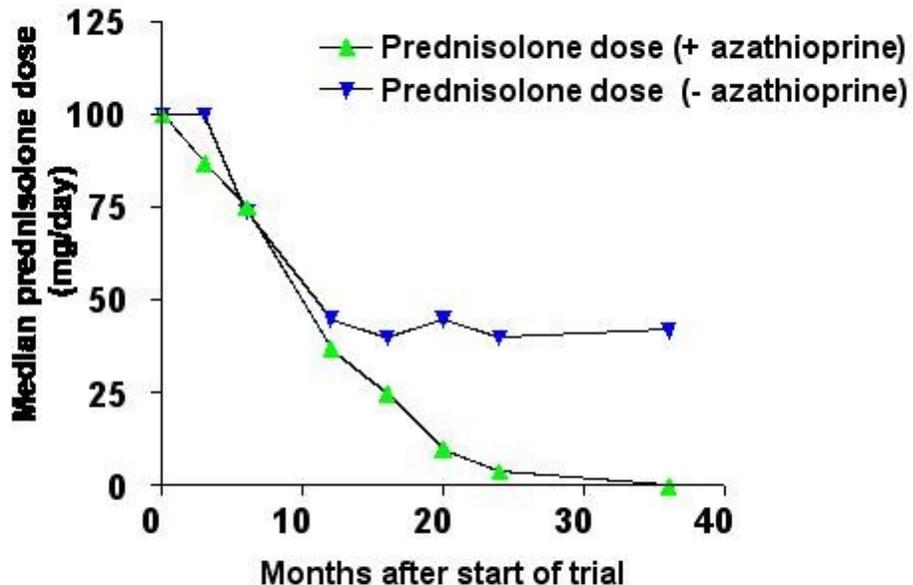
AChR antibody and clinical grades after different treatments for MG.

Thymectomy



Robb et al unpublished data

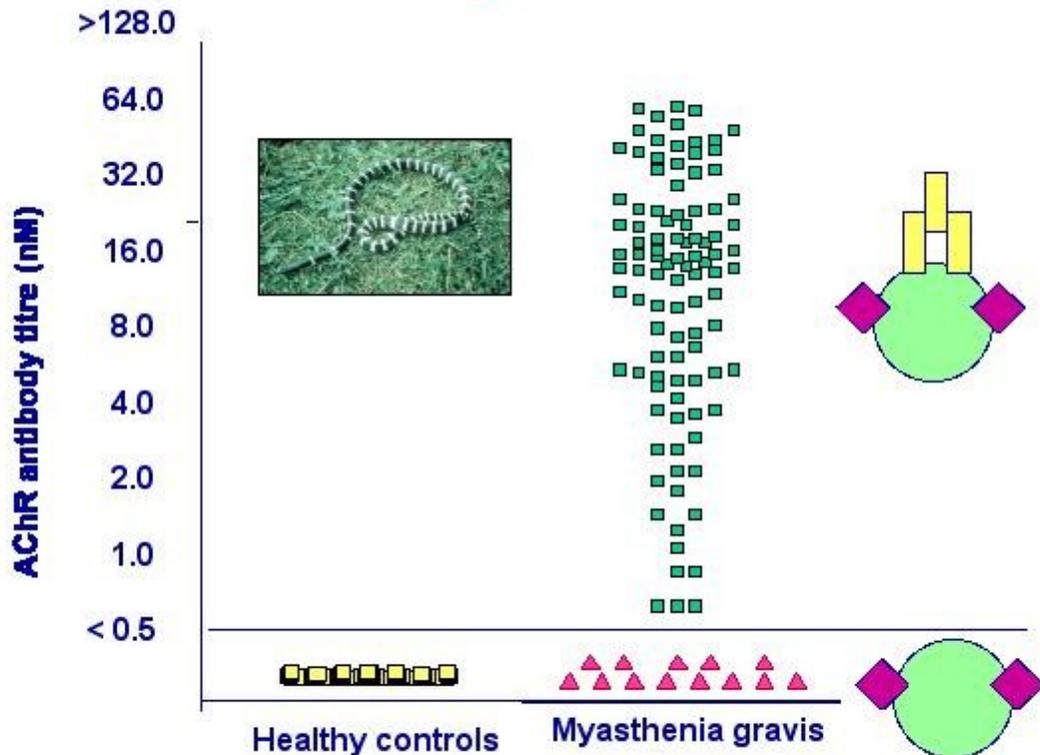
Prednisolone dose in patients with myasthenia with or without azathioprine



from Palace et al Neurology 1998: 50: 1778-1783.

Azathioprine is a steroid sparing drug but takes time to work

AChR antibody levels in MG



What about the patients who are negative?

Seronegative myasthenia gravis

About 15% of patients with typical symptoms of MG

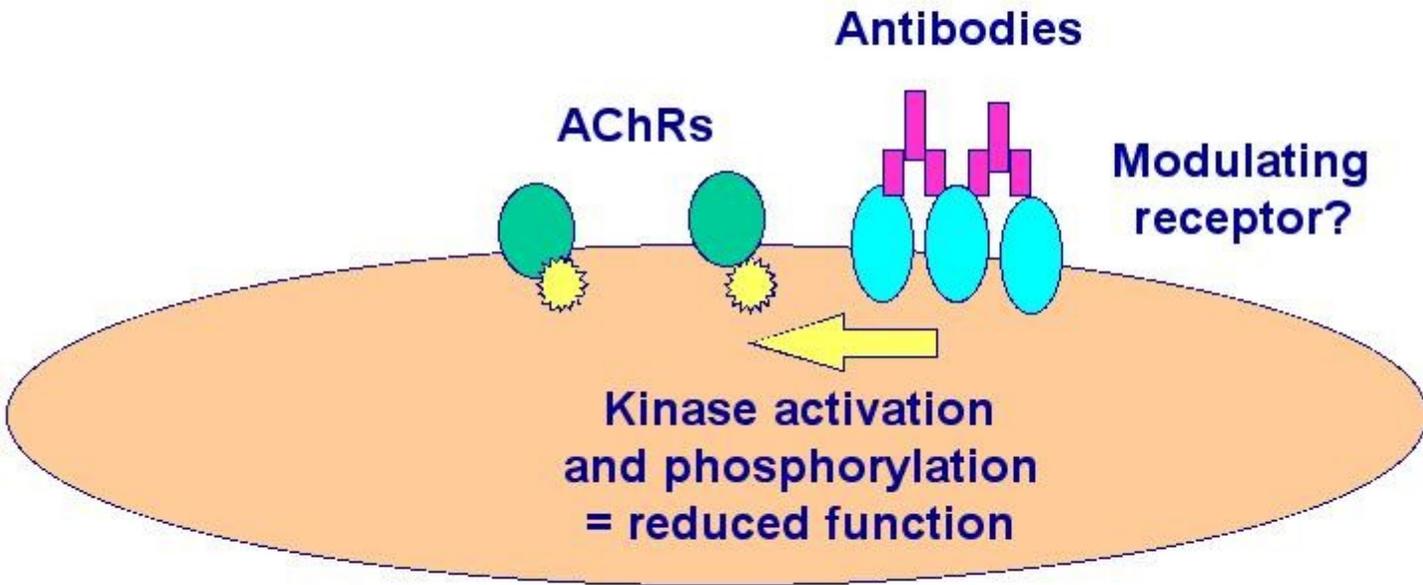
Patients respond to plasma exchange and immunosuppression

Injection of IgG into mice leads to defect in neuromuscular transmission

Functional assays

**Serum from seronegative MG patients
inhibits AChR function in a cell line**

Action of antibodies in AChR seronegative MG



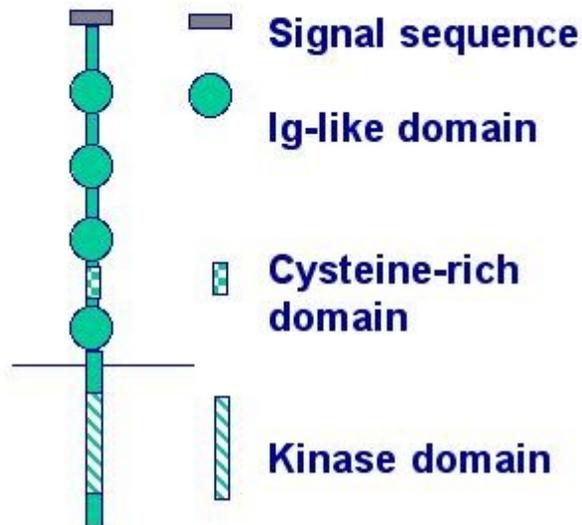
Paul Plested (Nuffield), Teresa Tang (MRC)

MuSK - a candidate antigen

MuSK (Muscle-Specific Kinase) is a receptor tyrosine kinase present in TE671 cells and at the neuromuscular junction

It plays an essential role in the agrin-dependent clustering of AChRs during development

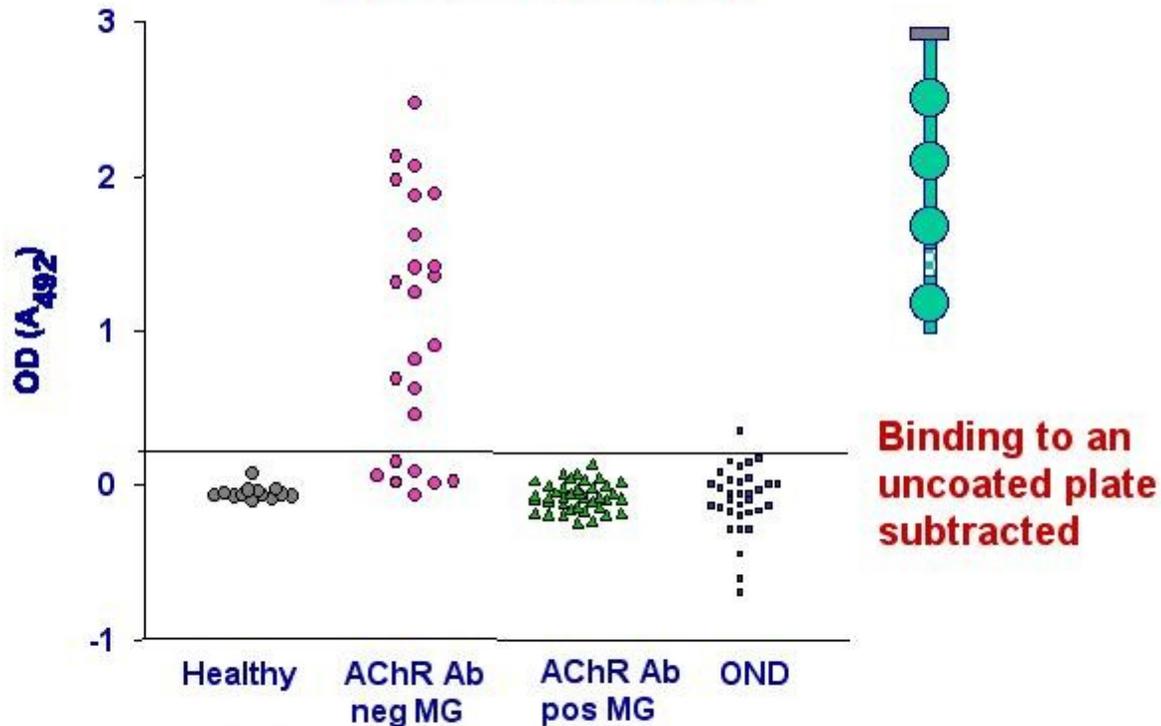
Role at adult neuromuscular junction not clear



Valenzuela et al 1995
De Chiara et al 1996
Hopf and Hoch 1998

ELISA assay using recombinant extracellular domain of MuSK secreted from a cell line

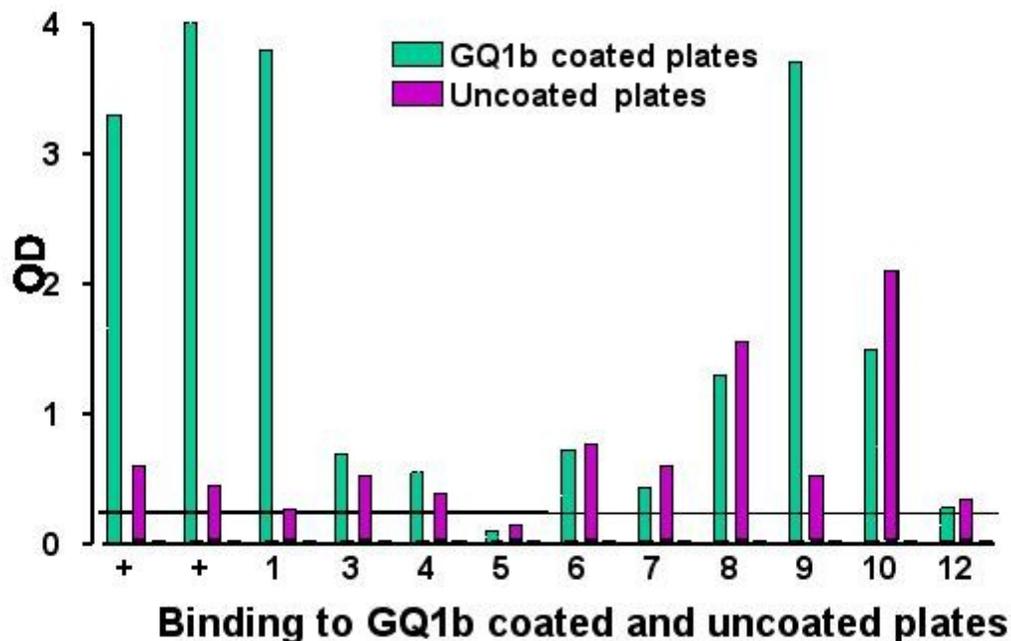
Binding of MG antibodies to extracellular domains of MuSK



Werner Hoch, John McConville (Wellcome)

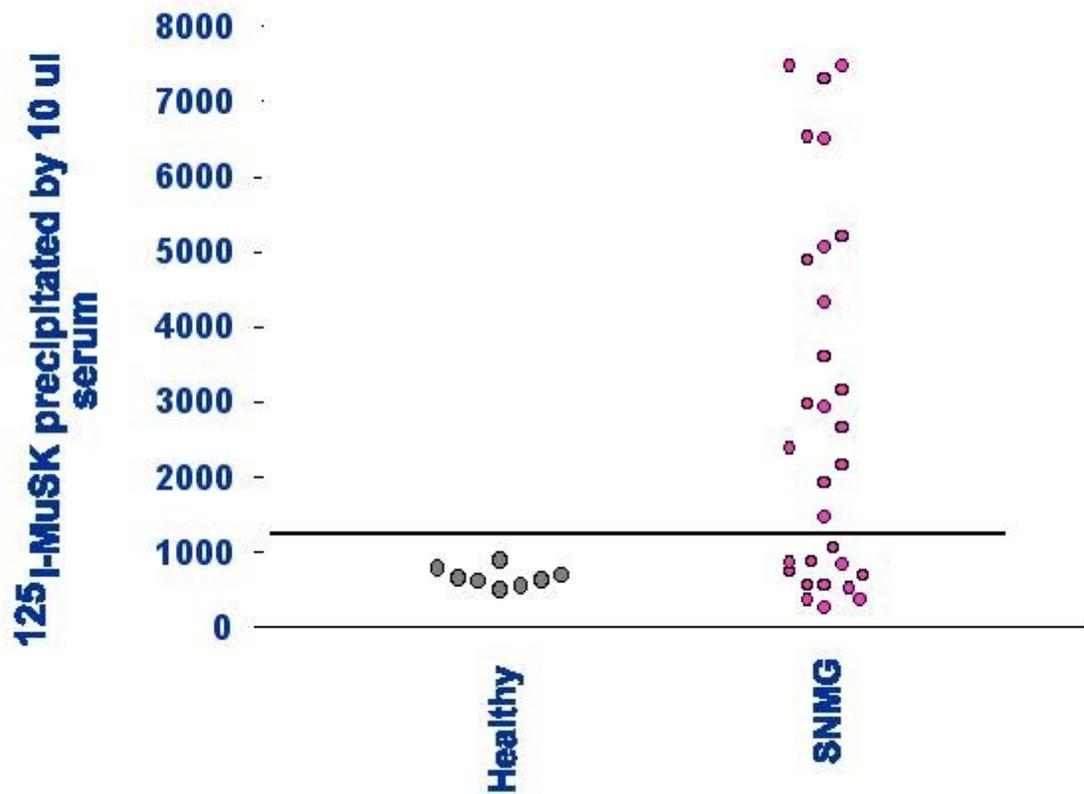
Why don't I like ELISAs

Binding to GQ1b ELISA plates
(1:100 dilution serum)

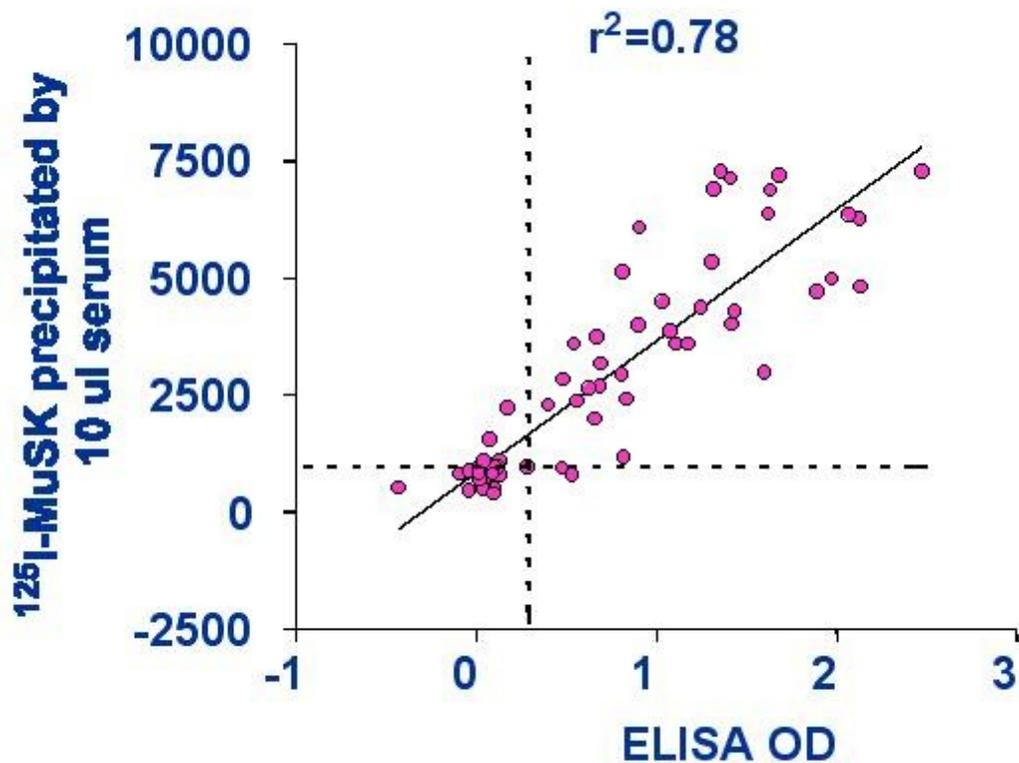


Radioimmunoprecipitation assay using purified recombinant extracellular domain of MuSK secreted from a cell line, and labelled with ^{125}I

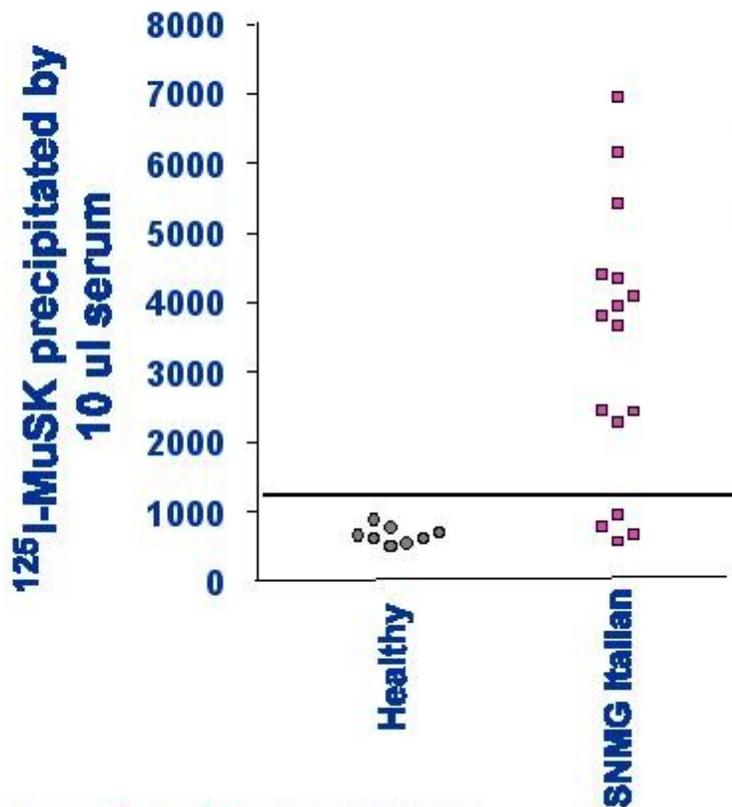
Immunoprecipitation of ^{125}I -MuSK (rat) by SNMG sera



Correlation between immunoprecipitation and ELISA



Immunoprecipitation of ^{125}I -MuSK (rat) by SNMG sera

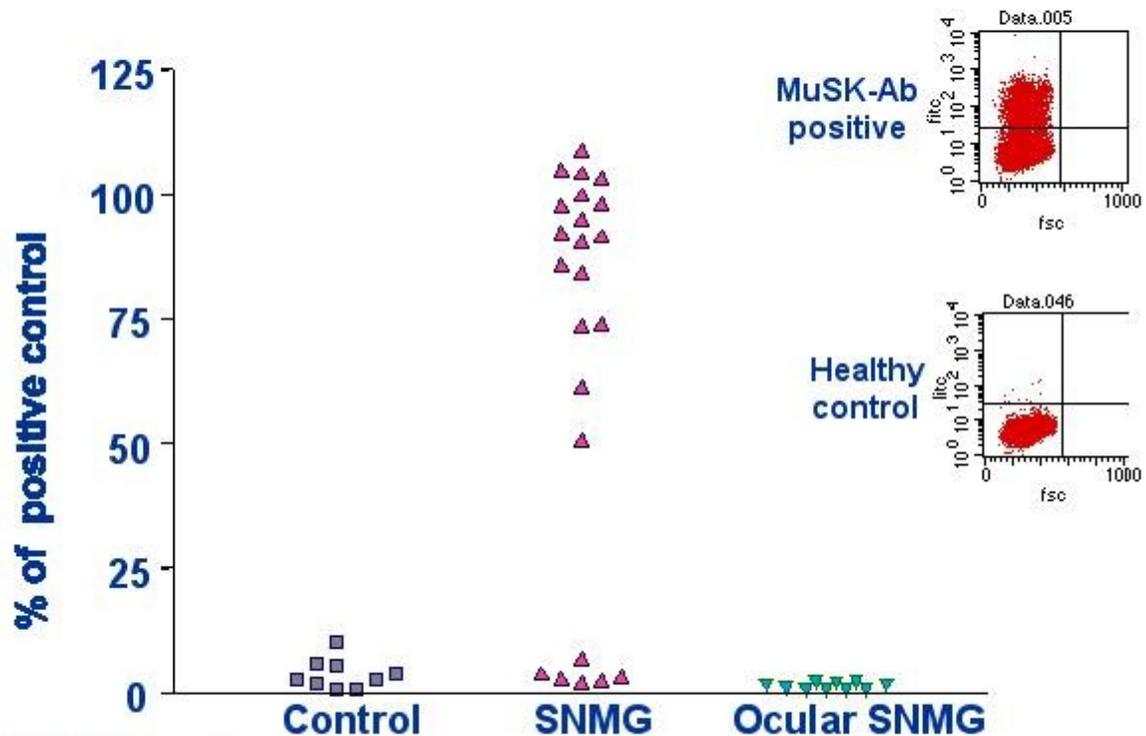


Even works in
Italian patients!

Sera from Amelia Evoli, Rome

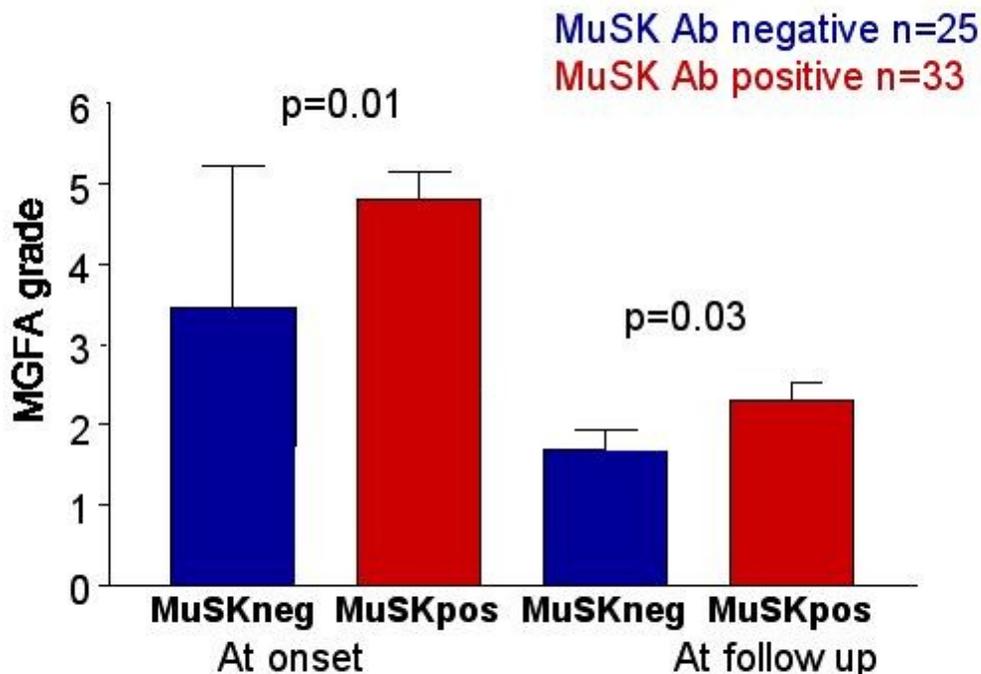
**Measuring antibodies by binding to
cell line expressing recombinant protein
using the FACS machine**

FACS analysis of binding of IgG to human MuSK-transfected HEK cells



John McConville

Are MuSK antibodies clinically significant?



SNMG patients with MuSK antibodies seem to have more severe disease at onset and be more difficult to treat

Preliminary data obtained from A Evoli in Rome and John Bowen in Oxford

MuSK antibodies in myasthenia

Present in 60% of AChR antibody negative

Not present in AChR antibody positive

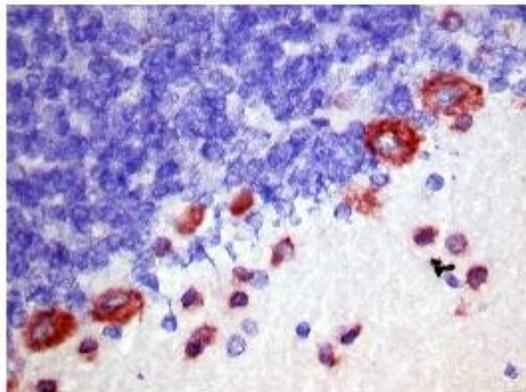
Seem to associate with more severe disease



Immunohistochemistry and western blotting for detecting antibodies to neuronal proteins

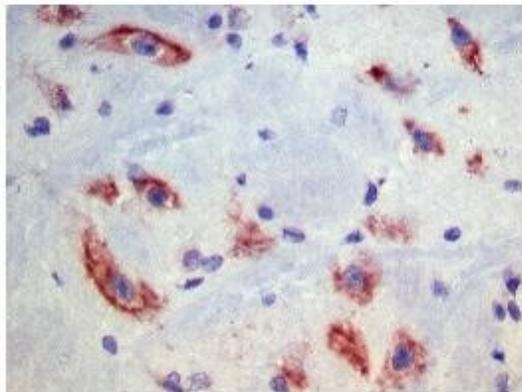
Anti-Yo Antibodies

Rat Cerebellum

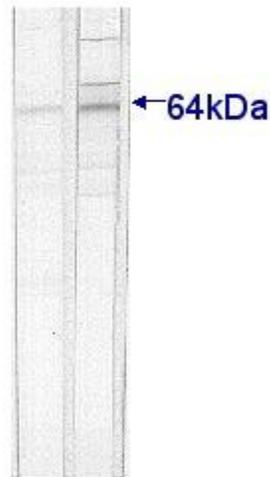


400x

Rat Brain Stem



400x



Immunohistochemistry is ideal for detecting antibodies to intracellular antigens
Is it useful for detecting other antigens?

**Can antibodies to CNS targets cause disease?
Neuromyotonia**

Cramps, pseudomyotonia, weakness, sweating

Spontaneous muscle activity of peripheral nerve origin

**Doublet, triplet or multiplet single unit discharges with
high intraburst frequency**

reviewed by Newsom-Davis and Mills 1993

Measuring antibodies to VGKCs

Use brain tissue as source of ion channels

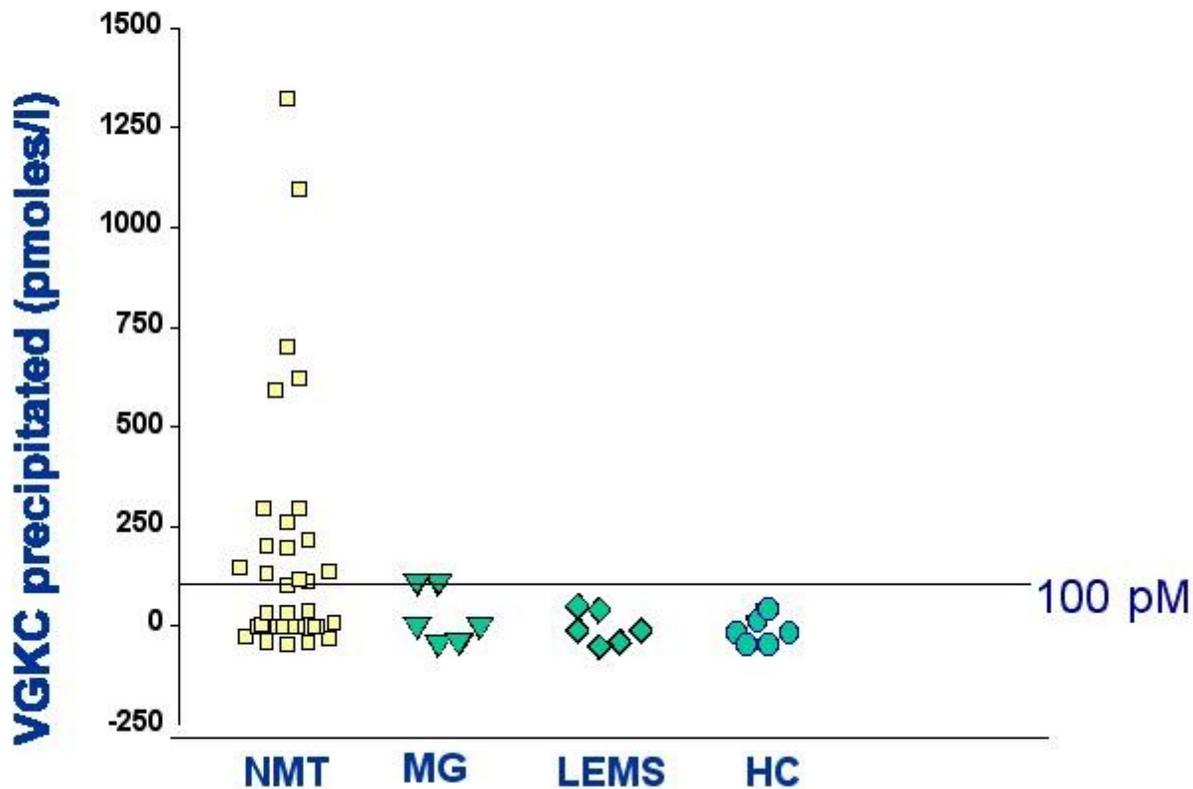
Dendrotoxin specific for VGKC

^{125}I -dendrotoxin

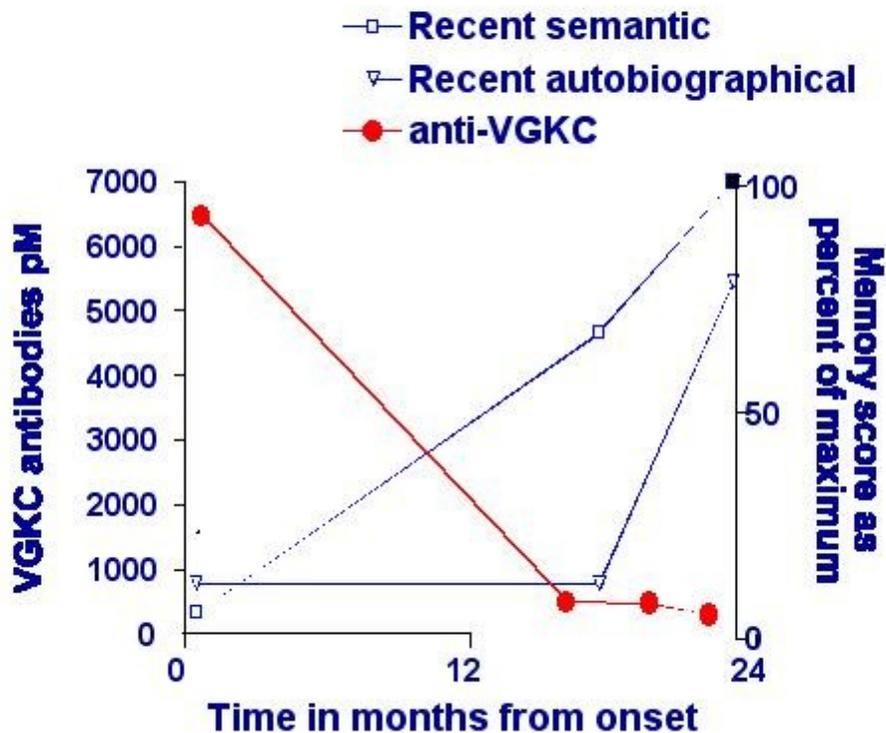


Measure immunoprecipitation of ^{125}I -dendrotoxin-VGKC complex by patient's serum

Antibodies to VGKC in acquired neuromyotonia



“Limbic encephalitis”: spontaneous fall in VGKC Ab correlates with improvement in memory tests



Comparing patient's antibody binding with antibody of known specificity

Patient with limbic
symptoms and VGKC
antibodies 6000 pM



Antibody to the Kv1.2
subtype of VGKC



Neuromyotonia with CNS symptoms

Morvan's syndrome (M1)

76 year old man

Muscle twitching, excessive salivation and sweating

Confusion, hallucinations, insomnia and excessive REMS
sleep

Constipation, cardiac arrhythmias

Liguori et al 2001

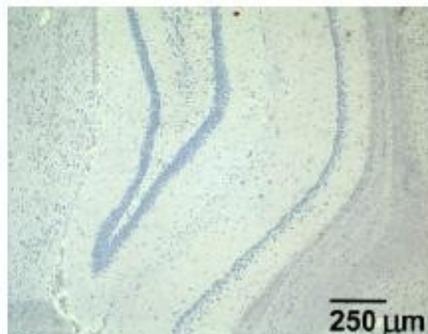
Studied at the University of Bologna and in Oxford

Morvan's syndrome

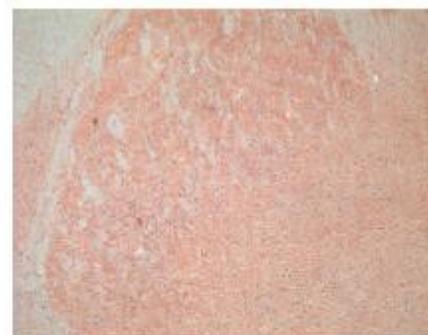
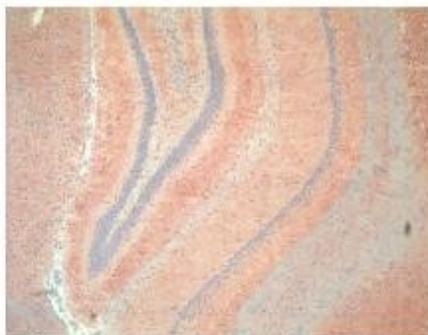
Hippocampus

Thalamus

Control
VGKC Ab
<100 pM



Morvan's
VGKC Ab
3000 pM



Binding to
hippocampus
and
thalamus

Indirect immunohistochemistry on rat brain sections

Neuromyotonia with CNS symptoms

Morvan's syndrome (M1)

76 year old man

Muscle twitching, excessive salivation and sweating

**Confusion, hallucinations, insomnia and excessive REMS
sleep**

Constipation, cardiac arrhythmias

VGKC antibodies very high

All symptoms responded to plasma exchange

Liguori et al 2001

Studied at the University of Bologna and in Oxford

Other patients studied in Oxford with
VGKC antibodies and

Seizures

Cognitive problems

Disorientation

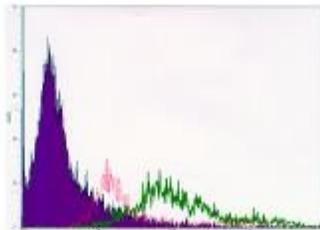
Hallucinations

Several have shown response to treatment

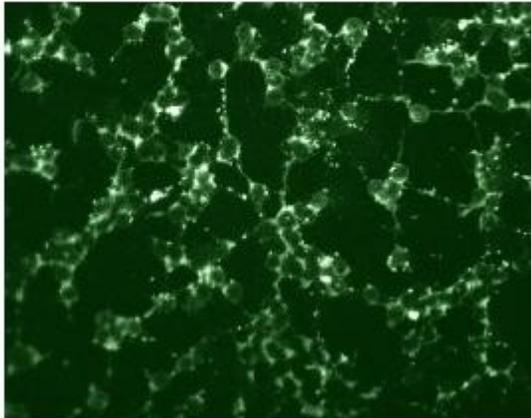
CNS disease can be associated with VGKC
antibodies and these patients tend to respond
to treatments or improve spontaneously

Are there antibodies to neuronal antigens in MS? using FACS to look for antibodies in MS

- **SKN cells grown as a monolayer in RPMI medium plus 10% FCS**
- **100,000 cells added to 20 microl serum in 200 microl medium (final concentration 1:10)**
- **Incubated on ice for 1 hour**
- **Wash x 3**
- **FITC conjugated anti-human IgG and IgM used at 1:50 and 1:25 respectively**



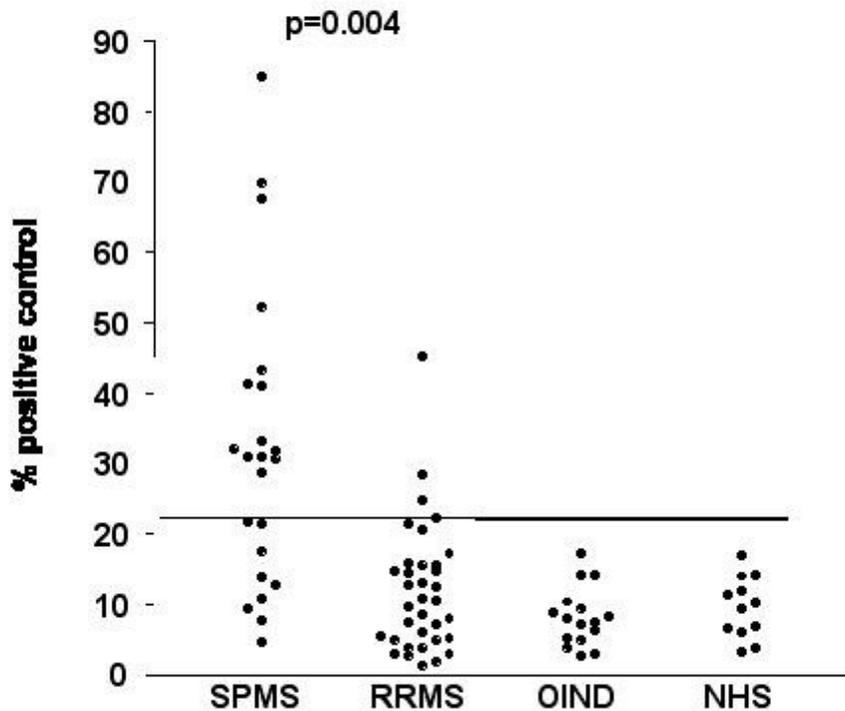
Oliver Lily, Jackie Palace



Immunofluorescence demonstrates binding
of MS antibodies to SKN cells but little to TE671 (muscle-like) cells

Oliver Lily, Jackie Palace

Binding of MS sera to SKN cell line
(mean normalised data from four experiments)



Spontaneous cytokine antibodies in myasthenia gravis

Myasthenia gravis occurs in three subgroups:

Early onset

Late onset

Thymoma-associated

Antibodies to IL12 and IFN alpha present
in patients with thymoma and MG

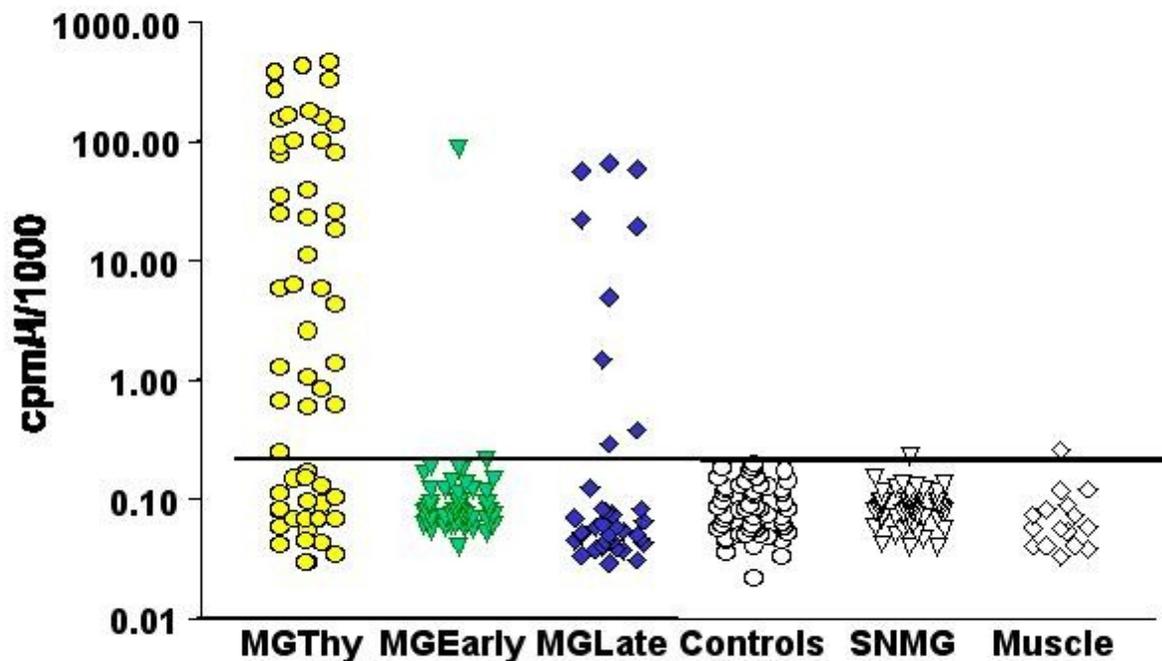
Meager et al Lancet 1997

Antibodies to cytokines

**Traditionally measured by inhibition of function
ie. neutralising antibodies**

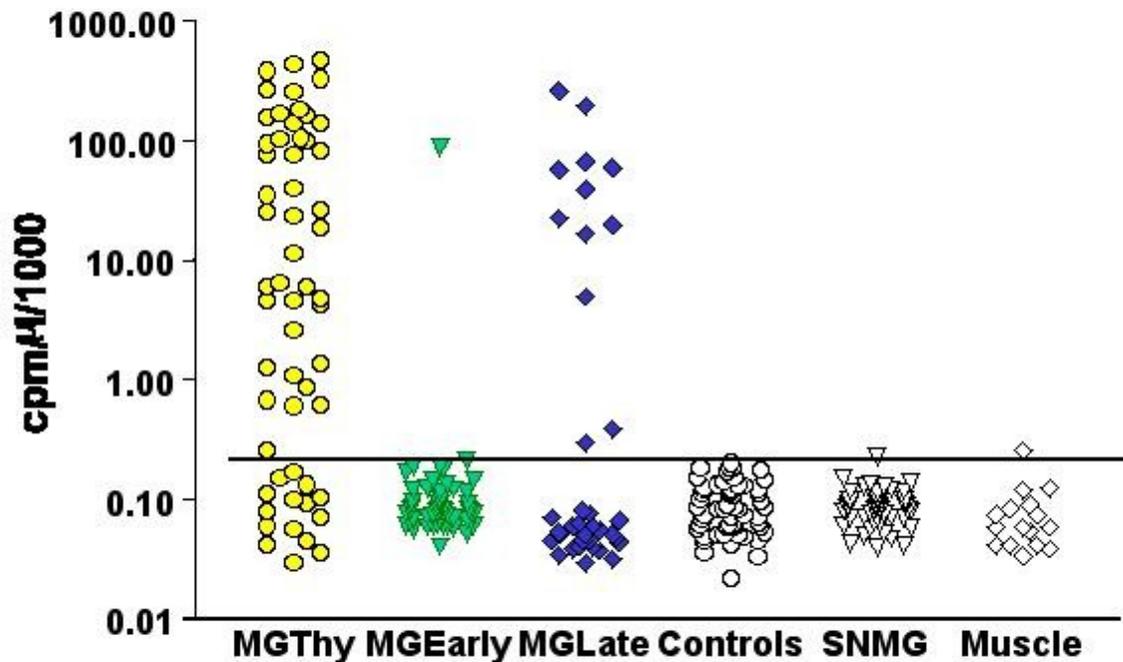
**Can be measured by immunoprecipitation
of ^{125}I -cytokine**

Anti-IL-12 antibodies in different groups of MG patients



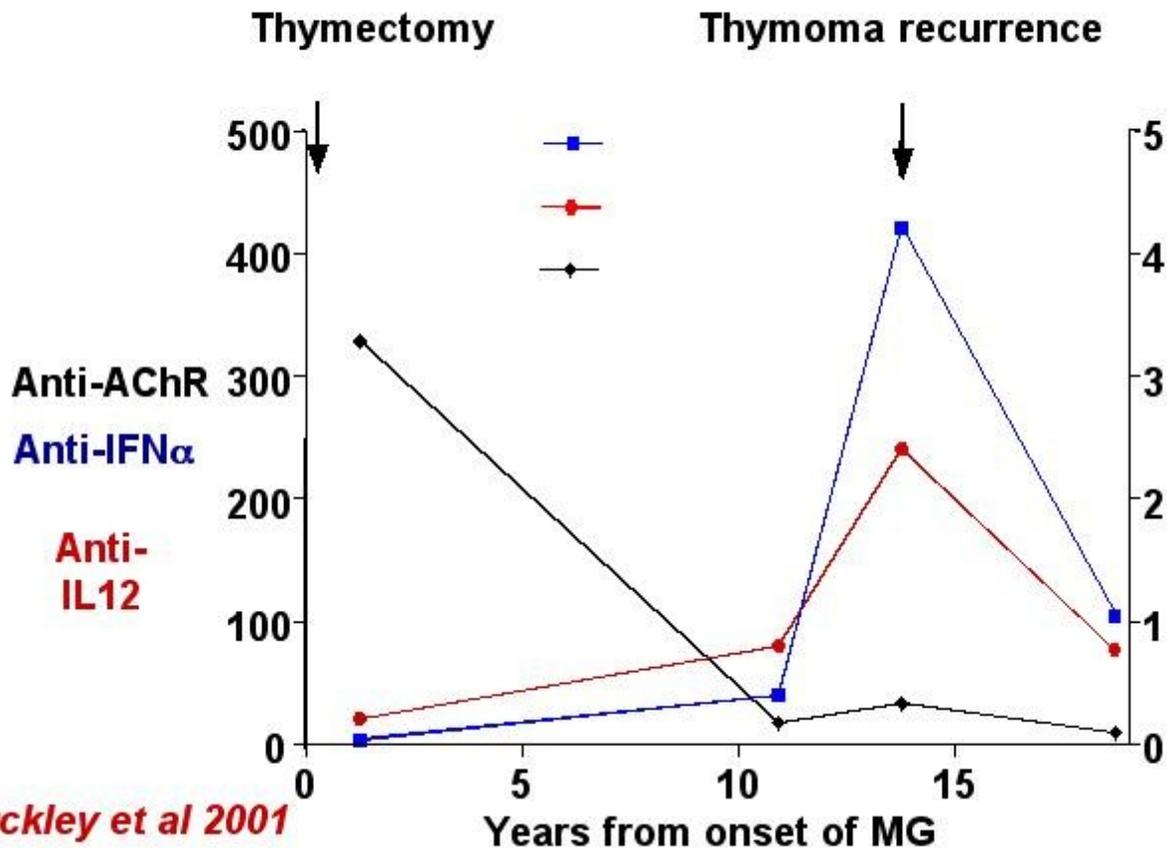
Buckley et al 2001

Anti-IFN- α antibodies in different groups of MG patients

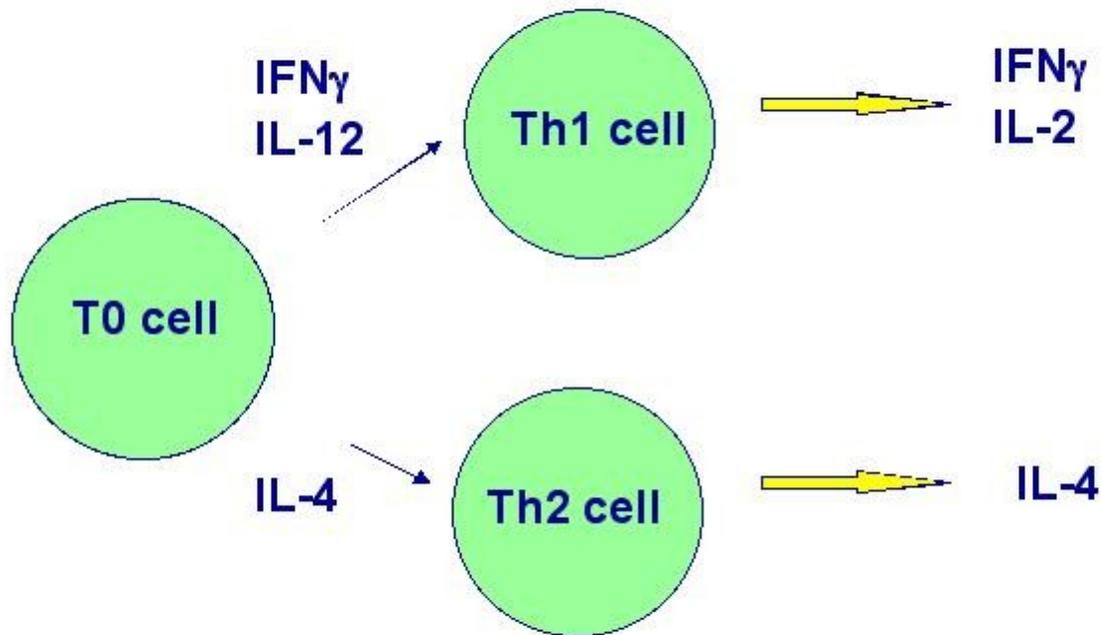


Buckley et al 2001

Cytokine antibodies indicate a tumour recurrence

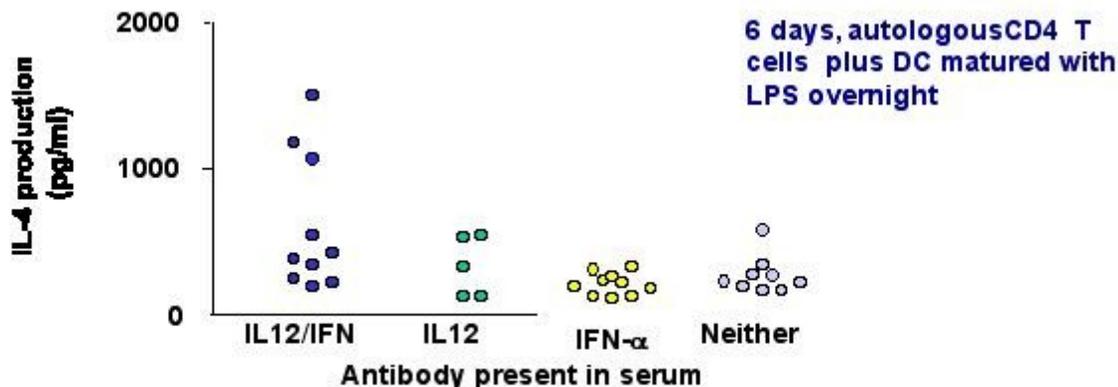
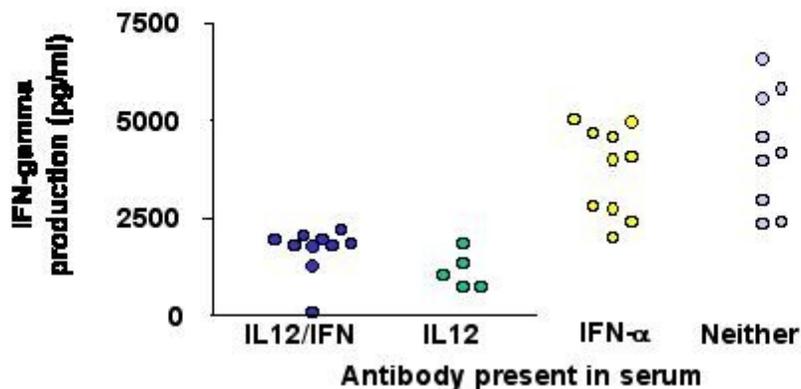


Cytokine secretion by T cells

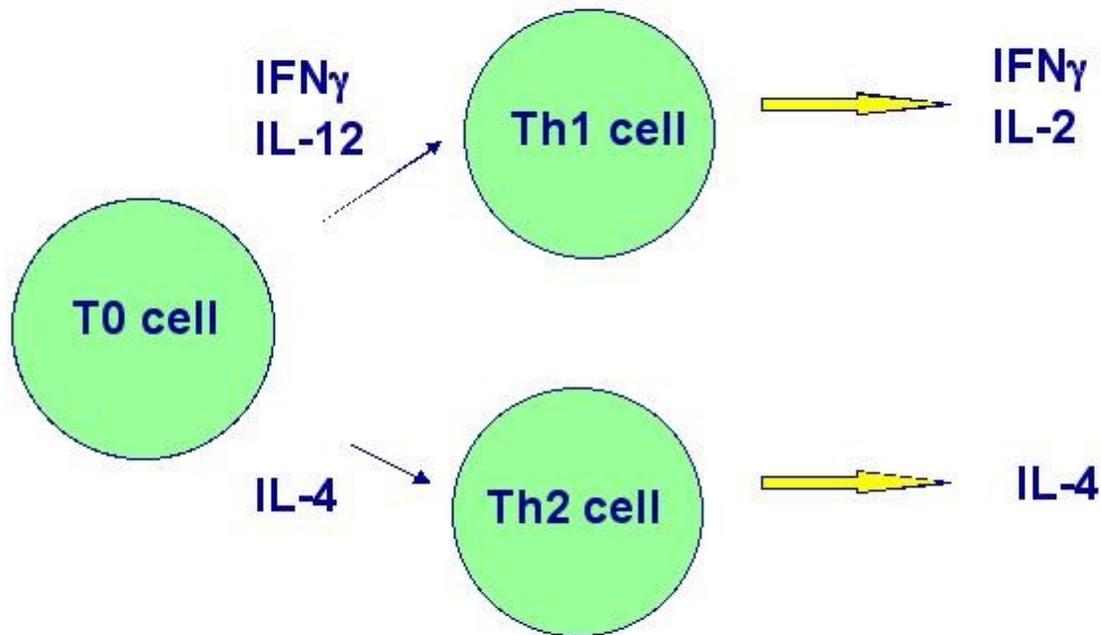


Antibodies to IL12 might prevent T0 \rightarrow T1

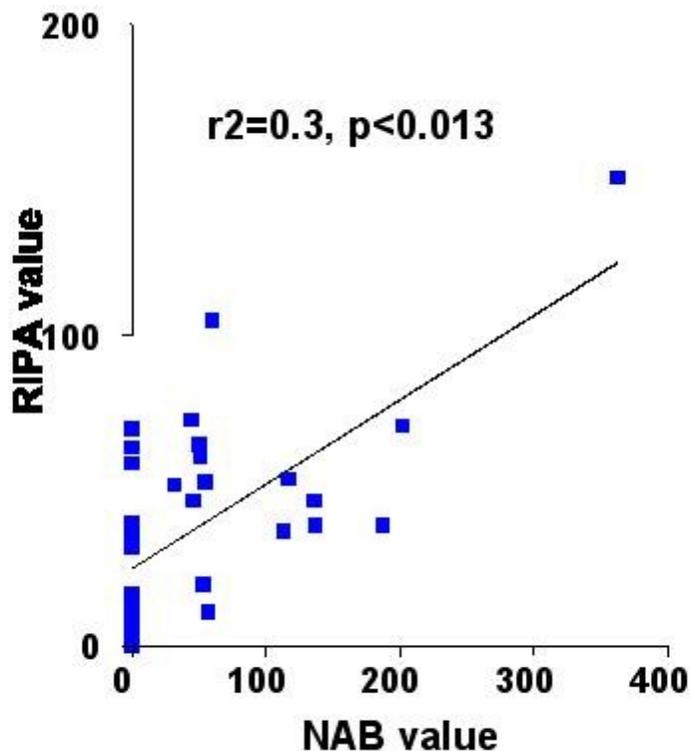
Influence of serum antibodies on T cell activity in vitro

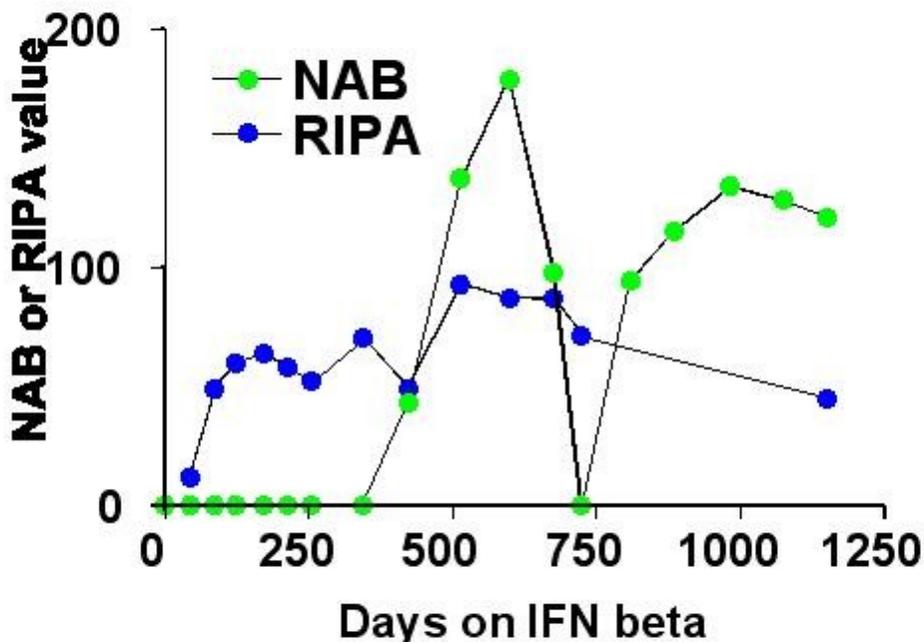


Cytokine secretion by T cells

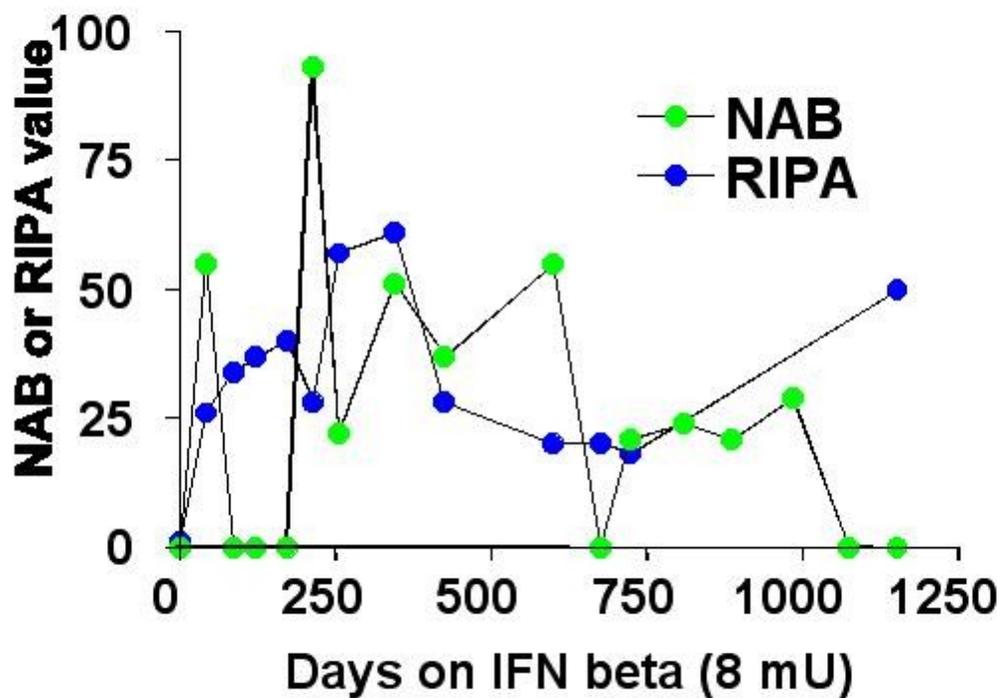


Antibodies to IL12 might prevent T0 \rightarrow T1

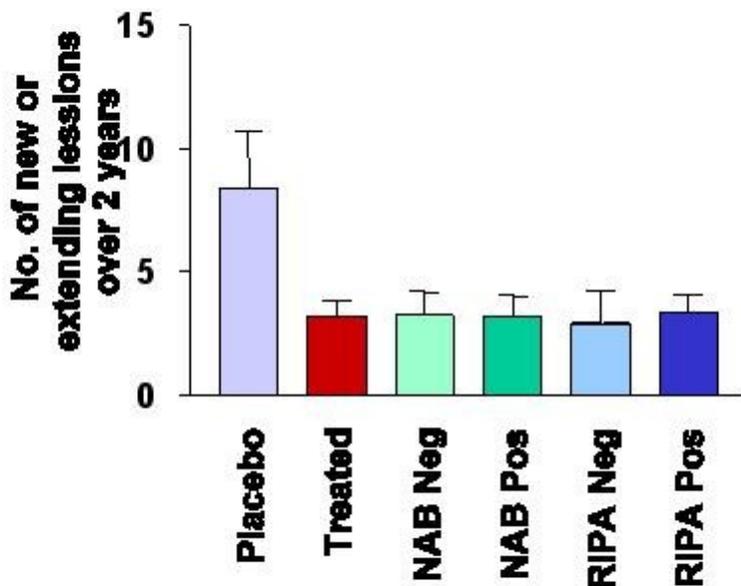




Antibodies to IFN beta measured by radioimmunoprecipitation are much less variable than neutralising antibodies



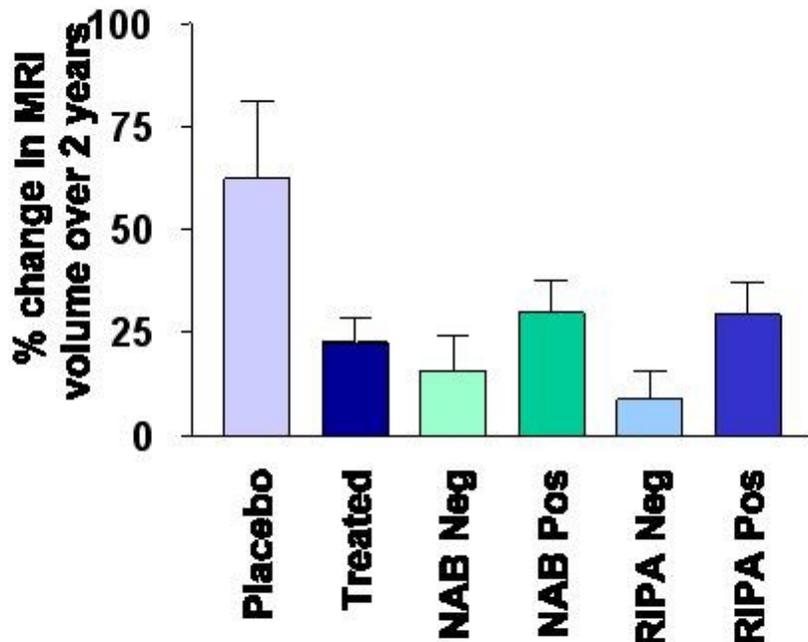
Do the antibodies predict lack of clinical efficacy?



Antibodies to IFN beta do not predict number of new or extending lesions during 2 years of treatment

Data from Joel Oger, Vancouver

Do the antibodies predict lack of clinical efficacy?



Antibodies to IFN beta do predict relative lack of change in MRI over 2 years of treatment

Data from Joel Oger, Vancouver

Summary and conclusions

Immunoprecipitation assays are very quantitative, sensitive and reliable

ELISA assays are useful if you have purified antigen, but non-specific binding to the plate must be considered

FACS can be a useful way of measuring antibodies – particularly if you don't know the antigen

Immunohistochemistry can be useful but mainly for antibodies that are markers rather than pathogenic

Functional assays are time-consuming and difficult to reproduce

Good assays DO produce meaningful results