Synaptic Autoimmune Disorders

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Learning Objectives

• Learn how our understanding of CNS autoantibodies has evolved over the last 40 years

• Recognize the syndromes associated with the various CNS synaptic autoantibodies
Autoimmunity at the NMJ

- Antibodies to the nicotinic acetylcholine receptor in patients with myasthenia gravis reported by Lindstrom and Patrick (1973)

- Animal model established in 1975 (Lennon, Lindstrom and Seybold)
Paradigm 1 (1970s-1980s)

Antibodies to neurons may target:

- Receptors or ion channels at the NMJ
  - Myasthenia Gravis (nicotinic acetyl choline receptor)
  - Lambert Eaton Myasthenic Syndrome (voltage-gated calcium channels)
Paraneoplastic Antibodies – Jermone Posner and Josep Dalmau

- 1980s-1990s
- Antibodies found in cancer patients with neuropathy, encephalitis, cerebellitis, etc.
- Antibodies target a variety of intracellular/nuclear proteins
- Antibodies are not directly pathogenic – immunization causes antibodies but no disease (Sillevis Smit et al., *Neurology* 45, 1873-8 (1995))

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Hu antibodies and a T-cell response targeting neurons

Pathology from patients with Hu antibodies has shown CD8+ T-cells targeting neurons.

In these pictures from Plonquet et al., T-cells are destroying DRG neurons.
Antibodies to intracellular antigens (Hu, Ma, Ta, CRMP-5, Yo)

- Strongly cancer associated
- Neuropathy, encephalitis, cerebellitis, etc.
- Poor outcomes
- Antibodies are not directly pathogenic – immunization causes antibodies but no disease (Sillevis Smit et al., *Neurology* 45, 1873-8 (1995))
- Rare – took 15 years to accumulate 200 cases of Hu antibodies (others rarer!)
Antibodies to neurons may target:

- Receptors or ion channels at the NMJ (pathogenic)
- Intracellular proteins in CNS and/or PNS neurons (not directly pathogenic) “onconeuronal antibodies”
  - Poor response to therapy
  - Targets antigens are not informative about symptoms
  - Strong cancer associations
Acquired Neuromyotonia “Isaacs’ syndrome”

• Findings:
  – Myokymia: undulating muscle movements
  – Pseudomyotonia: difficulty relaxing after contraction
  – Distal stiffness, cramps, and increased tone
  – Possibly muscle hypertrophy or hyperhydrosis

• EMG: spontaneous motor unit discharges, doublets, myokymic discharges, neuromyotonic discharges

• Activity originates from the motor axon
A 53-year-old man with myasthenia gravis (in remission) and prior thymoma resection developed diffuse myokymia, cramps, and muscle spasms over several weeks.

Needle EMG (at rest)

John Newsom-Davis (Oxford, 1990’s)

- Transfer of patients’ IgG to animals caused neuromyotonia
- Response to plasmapheresis, associations to thymoma, myasthenia
- Concluded an auto-immune basis was likely
- Well aware of PNS channelopathies – myasthenia gravis, LEMS
Newsom-Davis et al. (Oxford, 1990’s)

- Voltage-gated potassium channels (VGKCs) electrically stabilize axons.

- It would make sense if the antibodies disrupted VGKCs, just like the acetylcholine receptor is targeted in myasthenia gravis.

- How to detect the antibodies?
The radio-immunoassay for VGKC antibodies (Oxford, 1990’s)

- Prepare a slurry of brain proteins, including VGKCs

- Add $^{125}$I-α-dendrotoxin to label VGKCs

- “VGKC antibodies” can immunoprecipitate these labeled channels

- Measure radioactivity in the precipitate
VGKC complex antibodies associate with multiple phenotypes

• Isaacs’ syndrome
  – Many patients have VGKC complex antibodies
  – No other antibodies are strongly associated with peripheral nerve hyperexcitability
• Morvan’s syndrome
  – Also associated with VGKC antibodies
• Encephalitis
  – There are many types of autoimmune encephalitis characterized by antibodies (NMDA receptor, Hu, etc.)
  – VGKC is most strongly associated with thymoma

*How is this diversity of outcomes possible?*

Antibodies to neurons may target:

- Receptors or ion channels at the NMJ
- Intracellular proteins in CNS and/or PNS neurons
- VGKCs
  - With CNS and/or PNS symptoms
The identification of patients with CNS synaptic antibodies

- Dalmau et al. (2005) screened encephalitis patients for antibodies to brain or cultured neurons.
- These patients differed from those with antibodies to intracellular antigens.
Intracellular vs synaptic antibodies

Two types of CNS auto-antibodies

**Intracellular antigens**
- Hu, Ma, Ta, CVV2
- Strongly cancer associated
- Marker of a T-cell response killing neurons
- Syndromes relate to types of neurons killed - encephalitis, cerebellitis, neuropathy
- Poor prognosis

**Surface/synaptic antigens**
- Less cancer associated
- Much better prognosis – respond to immunotherapy
- Antibodies may have functional effects
- Syndromes mimic disruption of the target antigen
The identification of specific synaptic autoantigens

Immunoprecipitation and mass spectrometry indentified specific antigens.

The identity of target antigens can be confirmed by staining cells transfected to express that antigen – also makes a diagnostic test.
NMDA-R encephalitis

- Approximately 1500 patients since 2007
- Characteristic clinical syndrome
  - Psychosis, delusions, hallucinations, angry or agitated behavior
  - Seizures (variable)
  - Dysautonomia
  - Movement disorder (often complex writhing lingual/facial or limb movements)
  - Progressive catatonia, coma and respiratory failure
  - Association with ovarian teratoma
  - Relatively young demographic
  - Response to immunotherapy

Stages of NMDA-R encephalitis

A

Prodom 

Clinical worsening

- Agitation, psychosis, hallucinations, memory deficit, speech reduction, with or without seizures
- Abnormal movements, coma, respiratory failure, with or without dysautonomia

Clinical improvement

B

Corresponding PCP values:

- Pre: 0.01-0.2 μm
- Post: >0.2 μm

C

Ovarian teratoma in NMDA-R encephalitis

- Screen with transvaginal ultrasound, CT scan, etc.
- Atypical locations and bilateral tumors
- Neural tissue within tumor expresses the NMDAR and receptors are bound by antibodies
- Tumors are often small and easily missed

Sansing LH et al. (2007) A patient with encephalitis associated with NMDA receptor antibodies
NMDAR antibody mechanisms

- Antibodies reversibly deplete NMDARs from the dendrites of neurons
- Receptor cross-linking and internalization
- AMPAR antibodies have similar mechanisms
Management of NMDA-R encephalitis

Antibodies to neurons may target:
- Receptors or ion channels at the NMJ
- Intracellular proteins in CNS and/or PNS neurons
- VGKCs
- NMDAR
AMPA-R (GluR1/2)

- Limbic encephalitis
- Also associated with pure psychiatric manifestations (psychosis, apathy, aggression) or with language problems (expressive aphasia)
- Tumors (70%) of lung, breast, thymus

The GABA-B receptor

- Limbic encephalitis with prominent seizures, status epilepticus
- Relatively common among patients with LE and small cell lung cancer
- GABA-B mutations result in epilepsy
- GABA-B antagonists cause seizures

Boronat et al. Neurology 2011 Mar 76(9): 795-800
Glycine Receptor (Gly-R) antibodies

• Gly-R is the main inhibitory receptor in the spinal cord
• Associated with stiff person syndrome and hyperekplexia
• Progressive encephalomyelitis with rigidity and myoclonus (PERM)
• Syndromes resemble strychnine (Gly-R antagonist) toxicity
• Human Gly-R mutations – human startle disease
Paradigm 5 (2009-2010)

Antibodies to neurons may target:
- Receptors or ion channels at the NMJ
- Intracellular proteins in CNS and/or PNS neurons
- VGKCs
- Diverse synaptic receptors of the CNS (NMDAR, AMPAR, GlyR, GABA-B)
  - Excitatory or inhibitory
  - Ionotrophic or metabotropic
mGluR1 cerebellitis

- Acquired cerebellitis
- 4 cases since 2000, 2 with Hodgkin’s Disease
- mGluR1 critical for rapid cerebellar signaling
- Antibodies do not recognize mGluR5 (closest homolog)

mGluR5 and Ophelia Syndrome

• Ophelia Syndrome (psychiatric and memory disturbances with Hodgkins Disease)
• Rapid response to tumor treatment
• mGluR5 is critical for learning and hippocampal signaling
• No cross reactivity with mGluR1

Synaptic Autoantibodies

• Syndromes mimic pharmacologic or genetic disruption of the receptor
• Patients often respond to immunotherapy and/or tumor therapy
• Syndromes are variably associated with cancers
Paradigm 6

Antibodies to neurons may target:

- Receptors or ion channels at the NMJ
- Intracellular proteins in CNS and/or PNS neurons
- VGKCs
- Many (possibly EVERY) synaptic receptor
  - All sort of excitatory and inhibitory, ionotropic or metabotropic receptors
What about potassium channel antibodies?
Do VGKC antibodies recognize potassium channel subunit proteins?

• Preliminary reports (Kleopa et al., 2006) suggested “VGKC antibodies” recognize VGKC subunits.
• We attempted to confirm that dendrotoxin-sensitive VGKC subunit proteins (Kv1.1, Kv1.2, Kv1.6) were targets but were unable to do so.
• How is this possible?
The radio-immunoassay for VGKC antibodies (Oxford, 1990’s)

• The immunoprecipitation used for the RIA is “dirty”

• $^{125}\text{I-}\alpha$-dendrotoxin may be precipitated by antibodies to proteins that associate with VGKCs!

• What could these proteins be?
Leucine-rich glioma-inactivated 1 (LGI1)

- Secreted synaptic protein
- Associates with VGKCs and AMPA receptors via the ADAM proteins
- Mice mutant have seizures, early death
- Human mutations associate with temporal lobe epilepsy

Fukata et al., 2010
LGI1 antibodies: Clinical features

- Purely CNS Symptoms: 100% have encephalitis
  - 40% had myoclonus
  - 60% had hyponatremia
  - 82% had seizures
- Outcomes: 78% full recovery or mild disability
- Very few patients have tumors, not associated with thymoma
CASPR2

- Axonal protein important for proper localization of VGKCs
- Brain, spinal cord and peripheral nerve expression
- NOT synaptic
- Human mutations: autism, intellectual disabilities, schizophrenia, epilepsy
Caspr2 organizes VGKCs at the juxtaparanode of myelinated axons

Arroyo and Scherer, 2002.

Lancaster et al., 2011.
Caspr2 is an axonal protein that binds TAG-1 in cis and in trans to organize the juxtaparanode.
CASPR2 antibodies: Clinical features

- Symptoms:
  - Encephalitis AND/OR peripheral nerve hyper-excitability
  - Symptoms may occur in either order, months or years apart
- Good response to treatment, but may relapse
- Patients with VGKC antibodies and thymoma almost always are Caspr2 positive
- Other unusual symptoms and findings:
  - Overlap with MG (perhaps 15-20% have MG)
  - The only autoantibody of this type associated with pain
  - Peripheral neuropathy
  - Horner’s syndrome
Antibodies to neurons may target:
- Receptors or ion channels at the NMJ
- Intracellular proteins in CNS and/or PNS neurons
- Diverse synaptic receptors of the CNS
- VGKC-associated proteins
  - LGI1 (a secreted synaptic protein)
  - Caspr2 (a cell adhesion molecule that organizes VGKCs on axons, NOT synaptic)
  - Other targets not yet known
Thank you!

Mentors
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Coworkers
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• Meizan Lai
• Toby Ferguson
• Andrew Wong
# The disorders of synaptic autoimmunity

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Syndrome and unique feature</th>
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<tbody>
<tr>
<td>NMDA-R</td>
<td>Psychosis, seizures, dysautonomia, catatonia, Teratoma, young age group</td>
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<tr>
<td>LGI1</td>
<td>Limbic encephalitis, myoclonus, hyponatremia, VGKC antibodies</td>
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<tr>
<td>Caspr2</td>
<td>Encephalitis and/or neuromyotonia, VGKC antibodies</td>
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<td>GlyR</td>
<td>Stiffperson syndrome, hyperekplexia, PERM</td>
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<td>AMPA</td>
<td>Limbic encephalitis</td>
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<td>GABA-B</td>
<td>Limbic encephalitis with prominent seizures, status</td>
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<tr>
<td>mGluR1</td>
<td>Cerebellitis (+/- Hodgkin’s Disease)</td>
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<td>mGluR5</td>
<td>Ophelia syndrome</td>
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<td>More are yet to be discovered</td>
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The Ophelia Syndrome

• In 1982 Ian Carr described in his 15 ½ year-old daughter a reversible form of limbic encephalopathy associated with Hodgkin’s lymphoma (HL). He suggested that “perhaps a circulating neurotransmitter-like molecule secreted by the tumor” could cause the disorder that he named the “Ophelia syndrome”.

• 8 subsequent cases of reversible limbic encephalitis in the setting of HL have been reported

• We investigated the possibility these have an auto-immune etiology
The index case

46-year-old woman was evaluated for depression and personality change then seizures and worsening mental status. Medical evaluation revealed HL (stage IIIa). On examination she was alert but fearful and tremulous; she had a short-term memory deficit, delusions, and was emotionally labile.

**Brain MRI**: increased T2 signal in the mesial temporal lobes, cingulate gyrus, insular regions and right thalamus.

**CSF**: 23 white blood cells/ml (90% lymphocytes), total protein 55 mg/dL, glucose 57 mg/dL, and negative cytology.

**Labs**: negative for onconeuronal antibodies (Hu, Yo, Tr, CRMP5, Ma2, amphiphysin) and glutamic acid decarboxylase (GAD).

**Treatment**: AVBD chemotherapy, IV methylprednisolone followed by a slow steroid taper.

**Outcome**: Her seizures remitted and her mental status gradually improved to normal over several months. At her most recent evaluation (4 years after presentation), her mental status was normal.
Labeling of rat brain by patient’s antibodies (A) and a control serum (B)
Labeling of cultured hippocampal neurons by patient’s antibodies
Synaptic auto-antibodies

• Out patient’s anti-brain antibodies did not target the previously reported antigens:
  – NMDA receptor
  – AMPA receptor
  – GABA-B receptor
  – LGI1
  – Caspr2
  – Glycine receptor
  – mGluR1
Western blot of immunoprecipitate
Metabotropic glutamate receptor 5

- Similar to the other group 1 mGluR (85% amino acid homology to mGluR1)
- Critical for learning and memory (knockout mice with impaired learning)
- Important for LTD in hippocampus
Patient’s antibodies react with HEK293 cells transfec to express mGluR5

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<th>mGluR5</th>
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Patient with Ophelia Syndrome

Patient with cerebellitis

Control
But do not label HEK293 cells expressing mGluR1

mGluR1 Patient ab merge

Patient with Ophelia Syndrome

Patient with cerebellitis

Control
Validation with mGluR5-null tissue

Patient’s antibodies stain wildtype brain (A) but not mGluR5-null brain (B). Note the similar staining by a commercial antibody to mGluR5 with wildtype (D) and null (E) brains. Staining with serum from a patient with cerebellitis (C).
Conclusions

• mGluR5 antibodies associate with Ophelia Syndrome
• mGluR1 antibodies associated with cerebellitis with or without Hodgkin’s disease
• Diagnosis is important since both syndromes are treatable.
The missing “VGKC” antibodies

- In our series, only 2/18 patients with isolated PNH had CASPR2 antibodies.
- Only 5/7 with Morvan’s syndrome had CASPR2 antibodies (the other 2 were VGKC positive).
- The Oxford group (Irani et al., 2010) studied 100 patients with proven VGKC antibodies – 55 LGI1, 19 CASPR2, 3 VGKC, 18 unknown
- Clearly a substantial number of cases with VGKC antibodies – and with similar phenotypes without VGKC antibodies– remain unexplained.
Immunoprecipitation of the targets of VGKC antibodies

- CSF from a patient with “VGKC antibodies” label a cultured neuron.
- Immunoprecipitation and mass spectrometry identify the targets.
- Leucine-rich glioma-inactivated 1 (LGI1) and Contactin-associated protein-like 2 (CASPR2)
Patients’ antibodies react with LGI1 in isolation or (better) with its receptors ADAM22 and ADAM23.
Immunoabsorption with LGI1 abrogates reactivity of patients’ samples with brain

Incubate serially with control HEK cells (6 times)

Incubate serially with HEK cells expressing LGI1 (6 times)
Reactivity of patients’ antibodies with brain is abrogated in Lgi1-null mice
CASPR2 antibodies recognize CASPR2 in transfected cells.

Patient CSF
CASPR2
Merge

Patient Sera
CASPR2
Merge

Control CSF
CASPR2
Merge

20 µM
Can we be sure CASPR2 is a real target antigen in both brain and nerve?

- Immunoabsorption of patients samples with Caspr2 prevents reactivity with brain or peripheral nerve.
- Patients’ antibodies do not react with Caspr2-null tissues.