New Frontiers in Infectious & Autoimmune Encephalitis

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Recent Advances in Neurology
February 2018

Disclosures

Dr. Wilson reports: Research support to UCSF from Genentech

Dr. Gelfand reports research support to UCSF from Genentech, Quest Diagnostics, MedDay. He is PI of a National MS Society Institutional Clinician Training Award. Personal fees for medical legal consulting. Prior personal fees for consulting for Genentech.

Talk Outline

• Update on Encephalitis Epidemiology

• Conventional ID diagnostics

• New Frontiers in infectious diagnostics (metagenomic deep sequencing, other panels)

• Conventional autoantibody diagnostics

• New Frontiers in antibody discovery

• Update on AE Phenotypes, New Antibodies

• Q&A
Encephalitis is morbid and costly

UNITED STATES
- $2.0 billion USD U.S. encephalitis hospital charges 2010
- 280,000 U.S. hospitalizations 1998-2010
- 20,000 hospitalizations per year, 7.3/100,000 hospitalization rate
- 5.7% fatal, 10.1% if HIV/AIDS, 17.1% transplant-associated

ENGLAND
- 5.2-6 cases / 100,000 incidence

AUSTRALIA
- 5.2 / 100,000 hospitalization rate (1990-2007)

ITALY
- 5.9 / 100,000 hospitalization rate

George, et al. PLOS One, 2014
Khetsuriani, et al. CID, 2002
Granerod, et al. EID, 2013
Iro, et al. Lancet Infectious Disease, 2017
Huppatz, et al. EID, 2009
Iro, et al. Lancet Infectious Disease, 2017

Encephalitis trends in England over 30 years...

- Vaccine-preventable encephalitis has plummeted
- Encephalitis of Unknown Cause is on the rise

Encephalitis in the Early 21st Century - 2
Infectious, Paraneoplastic, Autoimmune, Unknown / Idiopathic?

<table>
<thead>
<tr>
<th>Year</th>
<th>Population</th>
<th>Infectious</th>
<th>Inflammatory / Autoimmune</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singh, et al.</td>
<td>2000-2012</td>
<td>52%</td>
<td>22%</td>
<td>26%</td>
</tr>
<tr>
<td>Pillai, et al.</td>
<td>2000-2010</td>
<td>Children</td>
<td>38%</td>
<td>34%</td>
</tr>
<tr>
<td>Granero, et al.</td>
<td>2000-2010</td>
<td>Children/Adults</td>
<td>24%</td>
<td>25%</td>
</tr>
<tr>
<td>Granerod et al.</td>
<td>2000-2009</td>
<td>Children/Adults</td>
<td>42%</td>
<td>21%</td>
</tr>
<tr>
<td>Mailles, et al.</td>
<td>2007-2010</td>
<td>Children/Adults</td>
<td>52%</td>
<td>Not sampled</td>
</tr>
<tr>
<td>Glaser, et al.</td>
<td>2006-2010</td>
<td>Children/Adults</td>
<td>36%</td>
<td>Not sampled</td>
</tr>
<tr>
<td>Pillai, et al.</td>
<td>2010-2012</td>
<td>Children/Adults</td>
<td>29%</td>
<td>8%</td>
</tr>
</tbody>
</table>

Encephalitis in the Early 21st Century Update: The Rise of Autoimmune Encephalitis

- Incidence in Olmsted County, MN 1995-2015:
  - Infectious Encephalitis: 0.8/100,000
  - Autoimmune: 1.0/100,000

Incidence is now similar between Autoimmune and Infectious (i.e., autoimmune no longer should be considered as rare)

- Autoimmune 1995-2005: 0.4/100,000
- Autoimmune 2006-2015: 1.2/100,000

3x increase in AE → Attributable to detection, new antibodies!

Dubey, et. al. Annals of Neurology, 2018

Emerging Infections

- Nearly half of 175 emerging infections are viruses
- 80% have a zoonotic source
- Highest concentration of emergence events
  - United States
  - Europe
  - Japan
  - Southeast Asia
- Rate of severe neurological symptoms caused by emerging viruses
  - 39% commonly do so
  - 10% rarely or occasionally do so

Examples of Emerging Infections in the U.S.

- HHV6 encephalitis in bone marrow transplant
- PML in HIV and other immunosuppressed patients
- Arenavirus (LCMV-like) in solid organ transplant pts
- Dengue virus in the Florida Keys
- West Nile virus
- Periodic measles, mumps outbreaks
- Neurologic complications of H1N1
- Chikungunya virus
- Zika virus
- Powassan virus

Tyler KL. Arch Neurol. 2009 August; 66(8): 939–948
Emerging Neurotropic Viruses: Global

- Toscana virus
- Tick-borne encephalitis virus
- Chandipura virus
- Bat lyssaviruses
- Monkeypox virus
- H1N1 influenza virus
- Poliovirus
- Enterovirus 71 (Cambodian outbreak)
- Nipah and Hendra viruses
- Japanese encephalitis virus
- Rabies virus
- Zika virus
- Chikungunya virus
- Ebola virus

Clinical Syndrome of Encephalitis

Infectious → Non-Infectious
Inflammatory

Other cause of encephalopathy

Autoimmune

Paraneoplastic

Clinical Syndrome of Encephalitis

Infectious → Non-Infectious
Primary Inflammatory

Other cause of encephalopathy

Neuronal Cell-Surface / Synaptic Antibody
- Usually responsive to immunosuppression

Clinical +/- Research based testing negative
- Variable response

Neuronal Intra-Synaptic Antibody
- Can be frustratingly refractory to conventional immunosuppression, though in minority may benefit identifying the cancer, when there is a priority
## Encephalitis: Classical “Infectious Disease” Definition

- **Inflammatory process of the brain with associated neurological dysfunction**
  
  Infectious Disease Society of America, Encephalitis Clinical Practice Guidelines, 2008

- **Encephalopathy >24 hours plus ≥2 of the following:**
  - Fever (within 72 hours of presentation)
  - Seizures (not fully attributable to a preexisting seizure disorder)
  - New focal neurological findings
  - Inflammatory CSF (pleocytosis)
  - EEG abnormalities indicative of encephalitis (excluding medication / metabolic effects)
  - Neuroimaging abnormalities indicative of encephalitis


## Why is it so challenging to pinpoint specific causes of encephalitis?

- >100 pathogens cause human encephalitis... and new autoimmune causes are being discovered!

- Infectious disease testing is limited by technical challenges, sample volume, cost

- Limitations and inefficiencies in antibody discovery – many likely autoimmune cases remain “antibody” negative. Challenging to prove non-antibody mediated pathologies (i.e. primarily T cell mediated processes, immune dysregulation, etc)
58 patients with encephalitis on brain biopsy at between 1983-2011, the final pathological diagnosis was "Encephalitis Not Otherwise Specified" (ENOS) in 49 (84%)

Clinical follow-up led to a more specific diagnosis in 6/19 (32%) with ENOS and 6/11:
- Primary CNS lymphoma (2), Rasmussen encephalitis (2), paraneoplastic (CV2/CRMP5 and Ma2), Listeria monocytogenes
- Clinical follow-up led to a more specific diagnosis in 6/11 (55%) w/o additional material: TB, HSV2, Toxo, bacterial abscess, LGI1 encephalitis, gliomatosis cerebri (on autopsy)

Even with brain biopsy, ENOS is the most common pathological encephalitis diagnosis. Better diagnostics are needed.
Zika Virus

• Phylogenetic analyses indicate Zika was introduced to Brazil in 2013
• 18 months before it was detected
• Nearly 2 years before recognized as a cause of microcephaly, meningoencephalitis and Guillain-Barré syndrome

Metagenomic Next-Generation Sequencing

• Analyzing all the genetic material in an environmental sample
• Massively parallel sequencing approach

Sequencing Library Prep

- Primer Design
- Extract DNA
- Make PCR Reaction
- Purify PCR Product
- Sequencing Library Preparation
- Hybrid Selection
- Sequencing
**Sequencing Library Prep**

<table>
<thead>
<tr>
<th>Step Sequence</th>
<th>Library Built</th>
<th>Library Indexes</th>
<th>Sequencing</th>
<th>Data Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA10420</td>
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**Abbreviations:**
- NT: nucleotide
- NR: non-redundant protein

**Sequencing Library Prep**

**Rare Pathogens**

**Novel Phenotypes**

Case 1

- 40 year-old physician with 15 years of relapsing myelitis and arachnoiditis
- Immigrated to US from India at age 22; lived in AZ, NY and MD
- No history of intravenous drug use
- No known animal, mosquito or tick exposures

Case 1

- 2002: Treated for TB meningitis for 3 months (had to stop due to abdominal pain)
- 2006: Non-diagnostic laminectomy at L5-S1
  -- 1 more year of anti-TB therapy
- 2015: valacyclovir + prednisone followed by...
  -- > 35 mg of daily prednisone plus...
  • Mycophenolate mofetil (up to 3000mg daily) for 8 months
  • Methotrexate (Jan 2017 to present)
  • Etanercept
  • Anakinra
Case 1

- mNGS of RNA extracted from CSF
  - 62,890 (1.1%) of the 5,750,572 sequence pairs were non-redundant, non-human
  - 1,493 (2.2%) of the 62,890 non-redundant, non-human sequence pairs aligned to the genus Taenia with a best match to Taenia solium
- Confirmatory qPCR and cestode antigen assay were markedly positive in the CSF
  - NIAID Laboratory of Parasitic Diseases (Dr. Theodore Nash)

Clinical Syndrome of Encephalitis

Infectious

Non-Infectious, Primary Inflammatory

Other cause of encephalopathy

Neuronal Cell-Surface /
Synaptic Antibody

- Usually responsive to immunosuppression

Clinical +/-
Research based testing negative

Neuronal Intracellular
Antibody

- Variable response

Clinical Syndrome of Encephalitis

Conventional testing for “Paraneoplastic” or
“Autoimmune Encephalitis” Antibodies?

Cell-Based Study

Stain against rodent brain slices

Culture dissociated hippocampal neurons (Rat)

Neuronal cell-surface

Best results if preserve native 3D conformation

Immunohistochemistry

Western blot
Evolving Paradigm of CNS Antibody Disorders

<table>
<thead>
<tr>
<th>Neuronal Intracellular (Classical Paraneoplastic)</th>
<th>Neuronal Cell-Surface/Synaptic (Autoimmune)</th>
<th>Astrocyte</th>
<th>Myelin</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRMP-5, Hu (ANNA-1), Yo (Purkinje cells), Ri, Mo, amphiphysin...</td>
<td>NMDA, VGKC-c/LGI-1 (Casp2, MAPK, GABA-B, DPFP...</td>
<td>AQP4 (NMO)</td>
<td>GFAP</td>
<td>A-beta (SICAS)</td>
</tr>
<tr>
<td>Usually associated with cancer</td>
<td>Sometimes cancer-related but some predominantly autoimmune, post-infectious</td>
<td>Variable (see text)</td>
<td>Not</td>
<td>Not</td>
</tr>
<tr>
<td>Often poor T-cell response. Antibodies are probably an epiphenomenon of tumoral autoimmunity</td>
<td>Antibody/complement-mediated with T-cell response</td>
<td>Not</td>
<td>Not</td>
<td>Not</td>
</tr>
<tr>
<td>Frequently poor response to immunosuppression</td>
<td>Usually good response to immunosuppression</td>
<td>Good</td>
<td>Good</td>
<td>Variable</td>
</tr>
</tbody>
</table>

*Note that Thyroid antibodies do not have a known antigenic target in the CNS.

Antibody Discovery Pipeline

- Rodent brain slice staining
- Immunoprecipitation → Mass spectrometry
- Programmable phage display
  - All possible 49 amino acid peptides from every protein encoded in the human genome with 24 amino acid overlap
  - Collection of phage each expressing 1 of these possible 731,000 peptides on the surface of their major capsid protein
  - Rapid, high-throughput, and quantitative assay for autoantigen discovery and characterization

Constructing the Peptidome

- All human proteins including those without amino acid overlap on HTP sequence identify
- Split into 49 amino acid peptides with a sliding window of 24
### Peptidome

- Generate Phage Library
- Immunoprecipitate Patient Antibodies and Sequence
- Phage display (M13 - helper phage) for proteins OX
- Phage binding of patient MAb to human protein on phage surface
- Immunoprecipitate for broad phage array screening
- Recover phage by RT-PCR
- Phage re-expression study followed by protein MS analysis
- Each protein diploidentic as human complex

### Case 2

### Evolving Paradigm of CNS Antibody Disorders

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<th>Neuronal Cell-Surface/Synaptic (Autoimmune)</th>
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<td>CRMP-5, Hu (JNANA-1), Yo (Purkinje cells), Ri, Ma, amphiphysin...</td>
<td>NMDA, VGKC-c/α2-1, LCAP, PK2, AMPA, GABA-A, GABA-B, DPPX...</td>
<td>AQP4 (NMD)</td>
<td>MOG (MS, sarcoidosis)</td>
<td>A-beta (SCID)</td>
</tr>
<tr>
<td>Usually associated with cancer</td>
<td>Sometimes cancer related but predominantly autoimmune, post-infectious</td>
<td>Variable (see footnote, may be T-cell)</td>
<td>Not</td>
<td>Not</td>
</tr>
<tr>
<td>Cytotoxic T-cell Response</td>
<td>Antibody/complement mediated with T-cell response</td>
<td>Antibody/complement mediated with T-cell response</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Frequently poor response to immunosuppression</td>
<td>Usually good response to immunosuppression</td>
<td>Good</td>
<td>Good</td>
<td>Variable</td>
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*Note that Thyroid antibodies do not have a known antigenic target in the CNS.
Case

- Meningoencephalitis +/- myelitis, rare disc edema
- CSF testing most sensitive (vs serum); "false" positive ~1% unaffected controls
- Radial perivascular enhancement
- Can have longitudinally extensive myelitis
- Can coexist with other antibodies (NMDAR, AQP4, VGKC, etc)
- Rx with glucocorticoids, MMF, AZA – but need better data

GFAP Autoimmune Astrocytopathy

MOG-Antibody Oligodendrocytopathy

- Optic neuritis, myelitis → "NMO-like"
- Parenchymal lesions, seizures, AMS → "ADEM"
- Esp in children (but also adults)
- Can be relapsing-remitting, esp with persistent high titer MOG antibodies
- IVIG responsive, also Rituximab, AZA, MMF; poorer response to injection MS therapies
- Only very recently commercially available in the U.S.
Anti-NMDAR Encephalitis – Updates

NMDAR Antibody Encephalitis - 1

- **Disease of the young** – 95% <45 years-old; 37% < 18 years-old
- **Characteristic Clinical Syndrome**
  - Prodrome (HA, fever, N/V, URI-like)
  - Acute neuropsychiatric symptoms
  - Amnesia, language dysfunction
  - Seizures
  - Abnormal movements
  - Subset with coma and autonomic dysfunction

NMDAR Antibody Encephalitis - 2

- **CSF** – Pleocytosis, OCBs; but can be normal
- **MRI** – usually normal or nonspecific
- **EEG** – slowing or epileptiform activity

- **Serology** – CSF or serum IgG NMDAR antibodies that target the GluN1 (NR1) subunit
  - 10% of pts will have +CSF serologies when serum is negative – send CSF when high index of suspicion!

- Ovarian teratoma (which has a component of neuronal tissue that can express NMDAR) in ~50% in women aged 12-45 – vaginal ultrasound and pelvic MRI
- Immunosuppression is favorable – OR 2.7 for better outcomes with aggressive treatment in largest series to date of 577 pts (Titulaer, Lancet Neurol, 2010)
Post-Infectious (post-HSV, VZV) NMDAR Encephalitis
Update: A Strong Case for Molecular Mimicry

-- NMDAR immunoreactivity will be found in 27%-30% post-HSV encephalitis patients if go looking
-- Some have clinical NMDAR encephalitis
-- Much less clear NMDAR immunoreactivity post-HSV means without clear clinical "relapse"
-- Also reported post-VZV but not yet post non-herpesvirae

One Brain, Two Specialties, Converging Mechanisms: AE

• **3% of new onset psychosis** patients with pure psychosis (otherwise indistinguishable to psychiatrists) had serum NMDAR antibodies in a prospective cohort (UK)
  - Lennox, et. al. Lancet Psychiatry, 2017

• **2% of consecutive postpartum psychosis** cases had serum NMDAR antibodies (Netherlands)

Are these true NMDAR "encephalitis" clinically or just immunoreactivity?
Better neurological phenotyping is needed

LG11, CASPR2, “Double-negative” VGKC – Updates
The VGKC-complex has several proteins that connect to or shuttle with the channel—
These are the actual antigenic targets in VGKC encephalitis.

- "Double-negative" VGKC (neg LGI1, neg CASPR2) often target cytosolic epitopes of Kv1 (potassium channel) or the radiolabel cofactor.
- CASPR2 IgG4 inhibits the interaction of CASPR2 with contactin, blocking could work therapeutically.

Anti-LGI1 encephalitis
Clinical syndrome and long-term follow-up

- CSF/MRI normal in 30%
- 90% of cases are autoimmune (not cancer associated)

"Double-Negative" VGKC (i.e. neg for LGI1/CASRP2) is much less likely to be pathogenic / clinically relevant

- "Double-negative" VGKC commonly binds to Kv1 potassium channels (but not neurons in slices or culture) or to the non-human alpha-dendrotoxin used in radiolabeling — both unlikely to be pathogenic.
- Clinical phenotype compelling for AE in only 28% of double-negative VGKC in a Netherlands cohort (i.e. 72% were not clearly inflammatory).
LGI1 Encephalitis – Evidence for Genetic Susceptibility

Netherlands:
HLA-DR7 in 88% of LGI1 (vs 19.6% of controls)
HLA-DRB4 100% of LGI1 (vs 46.5% of controls)

Korea:
HLA-DR7 in 91% of LGI1
HLA-DR4 in 73% of LGI1

Emerging Model
Environmental trigger (unknown) in genetically susceptible → AE


AMPA Antibody Encephalitis

- Limbic encephalitis – prominent amnesia
  - Median age 64 (i.e. favors older age of onset)
  - Cancer associated in the majority [64% in 1 series, 48% in another]
  - About half of pts have more than one antibody – value of panel based testing
  - Can have substantial atrophy and secondary neurodegenerative phenotypes

Hoftberger Neurology 2015
Joubert, JAMA Neuro 2015

Robust hippocampal staining
CSF of AMPA encephalitis pt

GABA-A Antibody Encephalitis

- Median age 40, but wide age range (2.5 months to 62 years)
- Seizures, movement disorders, multifocal WM lesions
- Post/Peri-infectious in children; tumor (esp thymoma) associations in adults
- Relatively rare (26 cases over 3+ years in Spanish series)

Hoftberger et al. Neurology 2013

GABA-B Antibody Encephalitis

- Median age 62 (range 16-77)
- Seizures, amnesia, AMS, rare opsoclonus-myoclonus or ataxia
- 50% SCLC/paraneoplastic
- Relatively rare (20 cases over 3+ years in Spanish series)

Hoftberger et al. Neurology, 2013
IgLON5 antibody-associated tauopathy: Neurodegenerative vs antibody mediated?

- Phenotypes:
  1) Sleep disordered breathing + parasomnias (NREM)
  2) Bulbar syndrome (dysphagia, stridor, dyspnea, sialorrhea)
  3) PSP-like syndrome
  4) Dementia +/- chorea

- Mean age 64 (range 46-83), M:F similar
- MRI may have atrophy but usually unremarkable; CSF bland in up to ~70%
- IgG4 predominates, usually both serum and CSF
- Striking HLA predominance (HLA DRB1*10:01 87%, 36X expected rate in population)
- Variable immune response (poor in European series but late start vs good in French/Mayo series)
- Tau on neuropathology -- primary vs secondary antibody response

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Extra Slides for Reference
Hashimoto's Encephalopathy (SREAT) vs Hashimoto's Thyroid Antibodies + some other cause of RPD

A clinical approach to diagnosis of autoimmune encephalitis

Lancet Neurology 2016

Diagnosis can be made when all six of the following criteria have been met:
1) Encephalopathy with seizures, myoclonus, hallucinations, or stroke-like episodes
2) Subclinical or mild overt thyroid disease (usually hypothyroidism)
3) Brain MRI normal or with non-specific abnormalities
4) Presence of serum thyroid (thyroid peroxidase, thyroglobulin) antibodies*
5) Absence of well characterized neuronal antibodies in serum and CSF
6) Reasonable exclusion of alternative causes

*There is no disease-specific cutoff value for these antibodies (detectable in 13% of healthy individuals)

"Steroid responsiveness" and +thyroid antibodies is neither specific nor diagnostic

Summary Approach to Empiric Immunosuppression for Autoimmune Encephalitis

ACUTE THERAPY
GLUCOCORTICOIDS +/- IVIG or PLEX

INDUCTION THERAPY (if very ill/severe)
CYCLOPHOSPHAMIDE +/- B-CELL DEPLETION (anti-CD20 therapy)

MAINTENANCE THERAPY (if concern for prolonged process or relapse)

Usually 1 of the following:
- Anti-CD-20 therapy, CYCLOPHOSPHAMIDE, AZATHIOPRINE, MYCOPHENOLATE MOFETIL, METHOTREXATE, CHRONIC STEROIDS
- Potential role for: tocilizumab (IL-6), bortezomib (proteosome inhibitor), TC19 or CD38 agents

Encephalitis and Antibodies to DiPeptidyl-Peptidase-Like Protein-6, a Subunit of Kv4.2 Potassium Channels
Annals of Neurology, 2015

- GI prodrome with intense diarrhea (the myenteric plexus is part of the nervous system and expresses this antigen)
- Hyperexcitability – seizures, myoclonus, exaggerated startle
- Encephalopathy

DPPX Encephalitis
Neurology, 2014