Inflammation in Lyme disease only affects locations that receive blood flow.

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CRBAI; Pocomoke, Maryland
Physician’s Round Table
3/15/2014
Tampa, Florida
Goals for today

- Ideally, this talk will help you put together data on inflammatory responses and brain structural data
- Chronic inflammatory response
- CIRS in differential diagnosis
- See how proteomics are now joined by **genomics**, a new generation of evidence of untreated illness
Last Three Round Tables

- CIRS
- Proteomics
- Genomics
- Added today are role of NeuroQuant, VIP and T regs in treating Post-Lyme
- Caution: If you ignore damp buildings many people will remain ill
Lyme: Infection or inflammation?

- Assumptions about infection = Ass²?
- Failure of LLMD to collate data fatal
- Lack of high quality studies comparing acute baseline to chronic;
- Comparing before/after antibiotics
- Comparing before/after biotoxin Rx
- Lack of careful differential diagnosis
Assuming Lyme is there

- What defense does that create to protect against mold illness (1)
- What defense exists to prevent colonization by MARCoNS (2)
- What defense does that present to protect against low T regs (3)
- How does Lyme prevent low VIP (4)
Assume Lyme is there-2

- If mold, MARCoNS, T regs and VIP weren’t even considered how can one ever say more antibiotics are needed?
- And if antibiotic failure is guessed at by symptoms/illness that 1-4 can bring, what basis is there to say co-infection(s) are untreated: NONE!
1-4 are incredibly common

- All treatable
- Easily diagnosed
- Sequential Rx shows benefit
- Learning T regs is not optional
- The Lyme world isn’t stuck in 2002 any more
Problems in Lyme

• What can one rely on to diagnose?
• Clinical? “Symptoms Impossible”
• Unusual symptoms unique? No way
• Culture? False positives: serious
• Western blot? Lab to lab incongruity
• How about physiology!
• There is a NQ fingerprint in the brain
Genomics will answer so much

- What we see in microarrays routinely involves elements never discussed
- Did you assay levels of peptidyl arginine/citrulline deimination Type VI?
- It sure can be a player
- And CD 86 before and after antibiotics? Start now, please
What kind of inflammation am I talking about?

• ESR, CRP? Nope
• Acquired immune responses? Nope
• Antibodies? No chance
• Innate? Absolutely; always look
• What do we call Th1, Th2, Th17, complement, coag, T reg cells, HIF, commensals all at same time?
• CIRS!
Is there a chronic* Lyme patient who isn’t CIRS (*ill > 6 months)

- To date, not in my experience
- Surely, there is a first time
- Is there a CIRS who isn’t Lyme?
- Yes, sir. Treat it!
- Once Lyme, does CIRS recurring later means Lyme is back?
- Absolutely not!
CIRS, a brief history

- Sepsis is the prototype acute SIRS
- What do we call the altered immune status of sepsis survivors?
- Post sepsis syndrome?
- How about multiple trauma, burns, pancreatitis too?
- Acute versus chronic time frame is one month
CIRS is systemic, interacting

- No way to say just one lab as source of fatigue, cognitive abnormalities, joints, respiratory problems
- All of the putative diagnoses have the *same final common pathway*
- You will see the same combo of multisymptom illness and labs
- Differential diagnosis key
All of these elements are blood-borne

- Agreed. How does a cytokine, TGF beta-1 or split product of complement know to stay in blood not visiting joint or nerve or muscle or lung or brain?
- Who would ever think that?
- IOM in 2004 said that inflammation only affected respiratory structures in patients affected by wet buildings!
Innate immune effects are systemic
Everywhere blood goes…

- Pre-formed proteins, ready to go
- Pattern receptors pick up foreign signals
- Activation is instantaneous
  - Imagine *what turns off* such activation
- Initiates acquired immunity from production of antibodies
- The names of innate immunity might be new: cytokines, complement, VEGF, TGF beta-1, MMP9, CD4+CD25++ (acquired)
Innate immunity isn’t new

- It was 1989 at the CSH Symposia
- Charles Janeway, looking at immunology, predicted an expansion of insight in innate immunity
- 1972 Lewis Thomas talked about the peculiar over-reaction of the host to toxins, thinking of bacterial toxins
- 1985 first description of TNF
- By 2000, over 50,000 papers published
- 2011 Nobel Prize, Bruce Beutler, Scripps
High cytokine levels in the capillaries attract white blood cells, leading to restricted blood flow, and lower oxygen levels. HIF stimulates VEGF and TGF B-1. Reduced VEGF leads to fatigue, muscle cramps, and shortness of breath (may be over-ridden by replacement with erythropoietin). TGF B-1 changes cell type and interacts with Treg cells.

Patients with certain HLA genotypes (immune response genes) may develop inappropriate immunity. Most common are antibodies to:

- Gliadin (affects digestion)
- Cardiolipins (affects blood clotting)

Treg cells: Pathogenic T cells

**Inflammation-related symptoms**

High levels of cytokines produce flu-like symptoms: Headaches, muscle aches, fatigue, unstable temperature, difficulty concentrating and more. High levels of cytokines also result in increased levels of several other immune-response related substances, including TGF B-1, MMP-9, IL-1B, and PAI-1. MMP-9 delivers inflammatory elements from blood to brain, nerve, muscle, lungs, and joints. It combines with PAI-1 in increasing clot formation and arterial blockage.

**Resistant Coag-negative Staph Bacteria**

Colonies of MARCoNS with resistance to multiple antibiotics may develop in biofilm or mucus membranes. The bacteria produce substances that aggravate both the high cytokine levels and low MSH levels.

**Reduced ADH**

Reduced MSH can cause the pituitary to produce lower levels of anti-diuretic hormone (ADH), leading to thirst, frequent urination, and susceptibility to shocks from static electricity.

**Reduced Androgens**

Reduced MSH can cause the pituitary to lower its production of sex hormones.
Biotoxin symptoms

- Fatigue, weak
- Ache, cramps
- Unusual, sharp, claw, electrical
- Light sens, red, blurred, tearing
- SOB, cough, sinus
- Abdominal pains, secretory diarrhea
- Joints, AM stiff
- Exec cognitive memory, concen, word, assimilation, confusion, disorien
- Mood, appetite, sweats, temp reg
- Thirst, pee, shocks
- Numb, tingle, taste
- Vertigo, tremor, skin
<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Controls</th>
<th>Cyano</th>
<th>WDB-1</th>
<th>WDB-2</th>
<th>WDB-3</th>
<th>PEAS</th>
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<td>Sweats (Night)</td>
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IOM strength of evidence

- Prospective, tightly controlled, risk
- Double blinded placebo controlled
- Meta analyses
- Case series
- Case reports
- Hypotheses
- Opinion
Cognitive and neurologic symptoms in WDB patients

- We call the illness CIRS-WDB*
- Systemic inflammatory responses
  - Blood brain barrier effects
- Innate immune activation
- Absence of regulatory neuropeptides
- TGF beta-1/T reg imbalance
- * Treating physicians expert report 7/2010; POA
Chronic cognitive abnormalities in CIRS-WDB patients

- Executive cognitive functions
  - Recent memory
  - Concentration
  - Word finding; assimilation of new
  - Confusion
  - Disorientation

NOT SPECIFIC FOR A GIVEN BIOTOXIN ILLNESS
Add to the list

- Absence of executive inhibition
- Tics
- Atypical seizures
- OCD
- What looks like depression
- What looks like anxiety/panic
- ALL REPORTED IN CAUDATE NUCLEUS ATROPHY SYNDROMES
Are these neuropsychiatric symptoms inflammatory?

- Seen in cases
- Not seen in controls
- Differences are $p < 0.001$
- Abate with Rx only (not self-healing)
- Recur with relapse (prospective!!)
- Genomics (mRNA, microRNA, antagomirs) will soon play key roles
- TGF beta-1, VEGF, MMP9, C4a key
How does a “Neuro-Naysayer” know otherwise?

- Data on thousands of patients affirms
- Data on treatment of thousands of patients
- No data from anyone (ever) to deny
- No research (ever) to deny
- The Naysayer has no data, no research, no prospective studies
- Countless mRNA markers affirm
- US Patent applied for 8/2013
No differences between CIRS-WDB and other biotoxin illnesses

- Mold (think water-damaged buildings)
- Dinoflagellates (Pfiesteria, ciguatera, Chattonella, ?? Karenina)
- Lyme, confirmed of course
- Apicomplexans (Babesia; labs in animals Sarcocystis and Eimeria)
- Cyanobacteria (Microcystis, Lyngbya, cylindrospermopsis)
Sx CLUSTER ANALYSIS

- Fatigue
- Weak, assimilation, aching, headache, light sensitivity
- Memory, words
- Concentration
- Joint, AM stiff, cramps
- Unusual skin sensations, tingling
- Shortness of breath, sinus
- Cough, thirst, confusion

- Appetite, body temperature regulation, urinary freq.
- Red eyes, blurred vision, sweats, mood, ice-pick pains
- Abdominal pain, diarrhea, numbness
- Tearing, disorientation, metallic taste
- Static shocks, vertigo
8 clusters are only seen in biotoxin illnesses to date

<p>| | |</p>
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<td>• History taken by the care provider</td>
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<tr>
<td>• No check lists</td>
<td></td>
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<tr>
<td>• No self reporting</td>
<td></td>
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<tr>
<td>• Use techniques of attorneys: multiple re-asking of same question to confirm reliability of history</td>
<td></td>
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<tr>
<td>• Physician history is a learned skill</td>
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Are symptom clusters in Lyme and biotoxin illnesses separate?

- No
- What takes away symptoms to then = controls?
- Sequential Rx, one step at a time
- Documentation at each step
- Absence of relapse with cessation of Rx
Lyme needing “ABXs only” is rare

- HLA and diagnosis: 22% are susceptible
- Follow a (+) HLA treated Lyme patient
  - What means treated?
  - Just symptoms? Not good enough
- Now getting worse
- LLMD says Lyme emerging from sanctuary (based on what?)
- CIRS says, “What is the new source of inflammation, given new labs?”
Defining what is wrong brings effective treatment

- Lowering levels of inflammagens: C3a, C4a, MMP9 and TGF beta-1
- Correct hormonal dysregulation
- Deal with auto-immunity
- Improve capillary hypoperfusion
- Eradicate commensal staphs
- Correct cellular immunity
What does treated CIRS mean

• Symptoms = controls
• Labs = controls
• Genomics = controls
• NeuroQuant = controls
  – No more caudate atrophy in CIRS-WDB
  – No more putamen atrophy in Lyme
• Re-exposure still brings relapse in three days in mold cases
What does cure of CIRS mean?

- Not sure we can use that term
- Absence of symptoms with re-exposure;
- And for completeness, does CIRS-WDB protect against Lyme? Nope
- Can one person have two sources of CIRS? Sure can and they often do
What is basis for so many divergent therapies

- Absence of collaboration
- Absence of a standard protocol
- Absence of a standard baseline
- Absence of a rigorous differential
- Absence of publication
- Absence of replication
- Acceptance of opinion as fact
True story about protocols

• 2010 POA Expert treating physicians report discussion
• “We want to publish your protocols”
• “I use protocols but I can’t tell you what they are since they are different for every patient.”
• Hmm, any variables not identified by this approach???
Objective Testing from Neurotoxicology

Visual Contrast Sensitivity (VCS)
- used over 40 years by US Air Force and in studies of non-biological toxicants
- Reproducible, reliable, portable, non-invasive, cheap!
- Just about the best marker beyond day 4 of biotoxin-associated/cytokine illness

VCS

High to Low Contrast

Low to High Spatial Frequency (Cycles per Degree of Visual Arc)
SBS Cases vs Controls

Visual Contrast Sensitivity

Spatial Frequency (Cycles / Degree)

- SBS Control Group 1 (N=239, Sx=1.8)
- SBS Control Group 2 (N=40, Sx=2.4)
- All SBS Cases (N=288, Sx=18.1)
- SBS Cases - Speciated (N=205, Sx=18.2)
- SBS Cases - Visible (N=78, Sx=18.1)
Controls Versus Initial Illness:
Fungi Genera Identified; Visible Evidence of Fungi Only; Water Damage Evidence Only.

Symptoms

Control 1.7
Fungal ID 18.8
Visible 18.3
Water Damage 17.1
Residential Cohort: Time Series

- Symptoms
  - Initial: 18.5
  - AC-1: 3.1
  - OROC: 2.0
  - BOC: 8.7
  - AC-2: 1.1
  - Prophyl: 2.1
Acute Lyme: All Cases Before Any Treatment & After All Treatment

Visual Contrast Sensitivity

Cases Before Treatment (N=20)
Cases After Treatment (N=20)

Spatial Frequency (Cycles / Degree)
## VCS in Lyme by step

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<tr>
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<th>Base</th>
<th>After Abx</th>
<th>After Rx</th>
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<tr>
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<tr>
<td>% Negative</td>
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<td>% Positive HLA Suscep</td>
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<td>% Positive HLA N-Suscep</td>
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### Logistic Regression Model - 8 Factor Score

**Combining Symptoms: Predicting Membership in the Group of Cases or Controls**

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<td>COLUMN TOTAL</td>
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**Percent Agreement** 98.85

Agreement Odds: 515/521
Disparities: 6
Standard Deviation: 0.47%

#### Confidence Limits

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#### Significance Test of Agreement

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<td>Pearson</td>
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Biotoxins are ionophores

- Forget relying on antibody testing if epitope separation isn’t confirmed
- Move from cell to cell
- Very small, many less than 1000 daltons
- Secreted against a gradient into bile by organic anion transport system (OATP)
- News: OATP is also found along blood brain barrier!!
Back to the brain

- Are executive symptoms telling us about abnormal brain structures?
- Brain physiology? Capillary!
- What we need is a dynamic imaging study that correlates with symptoms and physiology!
- And we have one: NeuroQuant
NeuroQuant

- Volumetric study of 11 brain regions
  - Can expand to 15
  - Changes over time key
- FDA cleared in 2007
- Software added to MRI of brain
- Takes 10 minutes ($96)
- Reproducibly reliable
- Controls data sets available
What do changes from normal in NeuroQuant mean?

- Changes in volume
  - Interstitial edema; increase volume
  - Atrophy or pruning; decrease volume
- Analyzed sequentially
- Correlate with clinical studies
- Correlate with genomics! (GFAP mRNA)
- We can link mRNA to changes in brain volumes with changes in clinical status
Review: what is the blood brain barrier?

- Endothelial cells and tight junctions
- Basement membrane
- Pericytes
- Astrocytes
- “The fence and the gate”
- Breached by VEGF, MMP9
- TGF beta-1 dual role
BLOOD BRAIN BARRIER

MMP9
VEGF
+ fluid
plasma proteins

TGF beta-1 → Gene Expression → GFAP

Adapted from: Karen Francis, Johan van Beek, Cecile Canova, Jim W. Neal and Philippe Gasque, The Blood Brain Barrier. Expert Reviews in Molecular Medicine, Vol 5, 23 May, 2003
What are we talking about when we say brain fog?

- Executive cognitive disruption from neuronal dysfunction?
- What injures the neuron? Toxin, infection, inflammation?
- How about pressure up or down?
- How about loss of dendritic connections ("pruning")
- Atrophy
Work with C4a and epo says the injury isn’t permanent

- 2006 study, CFS in Fort Lauderdale
- Certainly high C4a associated with high lactate = capillary hypoperfusion
- Correction of lactate resolved “fog”
  - C4a normalized
  - Cognitive symptoms normalized
- Of 8 measurements on MRS, cases were 5.2 before and 1.2 after
- Controls were 0.9
Dendritic pruning

- Hot topic in neurology
- Ranges from PTSD to MS
- (1) Loss of volume with pruning and then (2) replacement of lost volume with correction of inflammation
- Plasticity of dendritic connections
- What do we know about remodeling?
Glial fibrillary acidic protein

- Release from astrocytes after TGF beta-1 stimulation
- Effects can come from luminal and abluminal sides of BBB!
- Suppression neuronal re-growth
- Suppression reformation of axonal connections
Atrophy

- Loss of neuronal tissue
- Atrophy is permanent unless it is actually dendritic pruning
- How can one tell?
- MRS, NAA and creatine help
- Often we know only after a therapeutic trial
History of Structural Brain Imaging

- 1970s: Computerized tomography (CT) scans
A patient undergoing an MRI examination of the head.
FreeSurfer Methods

A. T1-weighted structural
B. Surface reconstruction
C. Inflation
D. Coregistration
FreeSurfer Methods

a) Inflation and spherification

b) Mapping to common space and comparison to brain atlas

c) Return with brain regions mapped
Typical MRI Slice
Segments Differentiated
NeuroQuant® Segmented Brain Image
NeuroQuant® Standard Report

Page 1

<table>
<thead>
<tr>
<th>Accession Number:</th>
<th>Referring Physician:</th>
<th>Exam Date:</th>
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<td>277625</td>
<td>ROSS, DAVID MD</td>
<td>2010/11/02 12:00:00 AM</td>
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MORPHOMETRY RESULTS

<table>
<thead>
<tr>
<th>Brain Structure</th>
<th>Volume (cm³)</th>
<th>% of ICV (3%-95% Normative Percentile*)</th>
<th>Normative Percentile*</th>
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</thead>
<tbody>
<tr>
<td>Hippocampi</td>
<td>7.71</td>
<td>0.51 (0.43-0.59)</td>
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<tr>
<td>Lateral Ventrices</td>
<td>18.66</td>
<td>1.23 (0.28-3.36)</td>
<td>41</td>
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<tr>
<td>Inferior Lateral Ventricles</td>
<td>1.13</td>
<td>0.07 (0.07-0.25)</td>
<td>7</td>
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</tbody>
</table>

AGE-MATCHED REFERENCE CHARTS*

![Graphs showing brain structure volume over age](image)
### MORPHOMETRY RESULTS

<table>
<thead>
<tr>
<th>Brain Structure</th>
<th>LH Volume (cm³)</th>
<th>LH Volume (% of ICV)</th>
<th>RH Volume (cm³)</th>
<th>RH Volume (% of ICV)</th>
<th>Asymmetry Index (%)</th>
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</thead>
<tbody>
<tr>
<td>Forebrain Parenchyma</td>
<td>530.42</td>
<td>33.41</td>
<td>544.54</td>
<td>34.30</td>
<td>-2.63</td>
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<tr>
<td>Cortical Gray Matter</td>
<td>238.78</td>
<td>15.04</td>
<td>239.66</td>
<td>15.10</td>
<td>-0.37</td>
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<tr>
<td>Lateral Ventricle</td>
<td>11.99</td>
<td>0.75</td>
<td>10.80</td>
<td>0.68</td>
<td>9.91</td>
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<td>Inferior Lateral Ventricle</td>
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<td>0.05</td>
<td>0.55</td>
<td>0.03</td>
<td>30.02</td>
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<tr>
<td>Hippocampus</td>
<td>3.98</td>
<td>0.25</td>
<td>4.02</td>
<td>0.25</td>
<td>-0.97</td>
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<td>Amygdala</td>
<td>1.98</td>
<td>0.12</td>
<td>1.95</td>
<td>0.12</td>
<td>1.32</td>
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<tr>
<td>Caudate</td>
<td>4.07</td>
<td>0.26</td>
<td>3.67</td>
<td>0.23</td>
<td>10.33</td>
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<tr>
<td>Putamen</td>
<td>5.23</td>
<td>0.33</td>
<td>4.30</td>
<td>0.27</td>
<td>19.55</td>
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<tr>
<td>Pallidum</td>
<td>1.31</td>
<td>0.08</td>
<td>1.32</td>
<td>0.08</td>
<td>-0.83</td>
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<tr>
<td>Thalamus</td>
<td>7.66</td>
<td>0.48</td>
<td>6.95</td>
<td>0.44</td>
<td>9.70</td>
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<tr>
<td>Cerebellum</td>
<td>65.25</td>
<td>4.11</td>
<td>66.08</td>
<td>4.16</td>
<td>-1.25</td>
</tr>
</tbody>
</table>

*The Asymmetry Index is defined as the difference between left and right volumes divided by their mean (in percent)*
Symmetrical Caudate Nuclei Heads
Radiologist vs. NeuroQuant®

<table>
<thead>
<tr>
<th></th>
<th>N positive/ Total N</th>
<th>% positive for atrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiologist atrophy</td>
<td>2/20</td>
<td>10%</td>
</tr>
<tr>
<td>NQ Extended atrophy</td>
<td>10/20</td>
<td>50%</td>
</tr>
</tbody>
</table>

Paired sign test, test statistic $M = -4.00$, $P=0.02$

Reference
Community Acceptance of NeuroQuant®

- NeuroQuant® is currently used in at least a dozen clinics and radiology centers across the USA:

<table>
<thead>
<tr>
<th>West</th>
<th>East</th>
<th>South</th>
</tr>
</thead>
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<tr>
<td>Santa Rosa Imaging Center</td>
<td>Lenox Hill Radiology &amp; Medical Imaging Associates</td>
<td>Virginia Institute of Neuropsychiatry</td>
</tr>
<tr>
<td>3536 Mendocino Ave., Suite 280</td>
<td>61 East 77th Street, New York, NY 10075</td>
<td>364 Browns Hill Court, Midlothian, VA 23114</td>
</tr>
<tr>
<td>Santa Rosa, CA 95403</td>
<td>East River Medical Imaging, PC</td>
<td>Center for Neurorehabilitation Services</td>
</tr>
<tr>
<td>Dr. James Brewer</td>
<td>519/523 East 72nd Street, New York, NY 10021</td>
<td>10710 Midlothian Turnpike, Suite 125, Richmond, VA 23235</td>
</tr>
<tr>
<td>University of California, San Diego, CA</td>
<td>Advanced Radiology</td>
<td>MRI CT Diagnostics</td>
</tr>
<tr>
<td>San Joaquin Community Hospital</td>
<td>888 Bestgate Rd, Ste 101, Annapolis 21401</td>
<td>4668 Pembroke Blvd, Virginia Beach, VA 23455</td>
</tr>
<tr>
<td>2615 Chester Avenue, Bakersfield, CA 93301</td>
<td>Washington Radiology Associates</td>
<td></td>
</tr>
<tr>
<td>Liberty Pacific Advanced Imaging</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16130 Ventura Blvd., Suite 100, Encino, CA 91436</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radnet, <a href="http://www.radnet.com">www.radnet.com</a></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sites in California</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
NQ algorithm
Cases versus control

- Look at differences between cases and controls
- Assign point value to 50%-99% of difference=1 and ≥100% =2
- Add the differences for controls; none are > 3
- Add the differences of cases; none < 4
Creating the mold NQ Index (MNQI)

- Now look at the sum of abnormalities for each measure between cases and controls
- Select top five $\geq 3.0$
- Return to the top five
- Create scoring on the top five
- Stratify scores by illness type
For CIRS-WDB, the five areas are not seen in any other illness to date

- Forebrain parenchyma increased
- Cortical gray increased
- Hippocampus increased
- Caudate decreased
- Pallidum increased
- **Cerebellum also enlarged
- **Thalamus and putamen normal
But for Post-Lyme

**Normal cortical gray, hippocampus and caudate**

- Small forebrain
- Small putamen
- Large thalamus
- Large cerebellum
Mold scoring system 0, 1, 2

- Forebrain $\geq 31.7$ and 32.3
- Cortical gray $\geq 16.4$ and 17.0
- Hippocampus $\geq .28$ and .30
- Caudate $\leq .24$ and $\leq .23$
- Pallidum $\geq .066$ and $\geq .071$
Lyme scoring system 0, 1, 2

- Forebrain $\leq 31.4$ and $\leq 30.9$
- Putamen $\leq 0.345$ and $\leq 0.335$
- Thalamus $\geq 0.55$ and $\geq 0.56$
- Cerebellum $\geq 4.25$ and $\geq 4.35$
No other combination like this in neurology known

- Selective increase in multiple areas
- Reduced caudate/putamen size but no other gray matter or basal ganglia
- Role of BBB in edema? Corrected by Rx illness!
- Caudate atrophy responds slowly to VIP (**); CIRS Rx lesser so (**)
- ** never reported anywhere
MNQI values in two cohorts

- Controls 2.5 and 1.9
- Untreated cases 6.6 and 6.4
- Partially treated cases 4.0 and 3.8
- Treated cases 2.0 and 2.1
- Relapse 7.0 and 6.5
- Cohort 1 N= 19
- Cohort 2 N= 65
So what is going on?

- Microscopic interstitial edema means loosening of BBB
- Caudate atrophy is not permanent!
- Pruning!
- How do we say that pallidum increases and caudate decreases yet no other posterior gray is altered
- Diffuse gray reduction is typical of MS, other CNS illnesses (Huntington’s chorea and PSTD)
Inflammation effects-1

- Looseing of blood brain barrier permits intrusion of peripheral elements into CNS
- Major players are TGF beta-1 (dual role), VEGF, MMP9
- Capillary permeability
- OATP!!
Inflammation effects-2

- The new buzz phrase is “TH17/Treg imbalance”
- Rising TGF beta-1 will direct T regs into tissue to suppress inflammation if there is ROR receptor present but if not, and IL-6, IL-17, IL-23 are, the Tregs are converted into pathogenic T cells that make more TGF beta-1
Inflammation effects-3

- T regs act in the brain
- the T reg imbalance can be from systemic T regs (thymus derived)
  - CD4+CD25++127 lo/-
- Or it can be from acquired T regs
  - CD4+CD25++
  - The days of looking at CD4+CD25+ are over (that was last years buzzword)
• DNA is sitting in forest of acidic proteins, slathered with histones
• Oh, this matrix lets one gene or another become activated?
• And how does a gene get suppressed?
• Remember, 98% of DNA is “just junk”
Genomics-2

- mRNA is just the messenger, boys
- microRNA shoots it anyway
  - Up to 100 kinds of mRNA
  - Gorgeous treatment potential
- Antagomirs block microRNA
- 1955 Watson Crick model was a good start but not wholly correct
Central Dogma of Molecular Biology

- **DNA**: Highly stable
- **RNA**: Highly unstable
- **Protein**: Moderately stable
Functional Genomics

**STIMULUS**
- Infections
- Toxins
- Wounding

**RESPONSE**
- Immunity
- Stress
- Tissue clearance

**Gene expression**
- Monitor transcriptomic variation using a **microarray**
  - *Transcriptome* = set of all RNA molecules produced by the genome at any one time

- transcriptomes are sensitive indicators of both disease status and emerging health hazards
Gene Structure

Coding regions account for 2% of the $3 \times 10^9$ bp in the genome

Approximately 20,000 - 25,000 genes in the human genome
Approximately 100,000 coding transcript variants
These transcripts are easily measured by DNA microarrays
Transcriptomics of CIRS-WDB

• Whole genome microarrays were run on 100 subjects (patients plus controls) to measure the transcriptome of a whole blood sample.

• 150 genes in males and 180 genes in females were identified as dysregulated and used to build a CIRS-WDB gene panel sufficient to produce a 90% success rate in identification of illness by using a Support Vector Machine classification algorithm.

• Many of these genes support systems found dysregulated at the protein level in these same patients, such as complement and coagulation pathways.
CIRS Panel Genes

The genes responsible for antibody production are very active; All the following are up-regulated in patients.

- Immunoglobulin heavy constant alpha 1
- Immunoglobulin heavy constant gamma 3
- Immunoglobulin heavy constant gamma 1
- Immunoglobulin heavy constant delta
- Immunoglobulin lambda variable 3-10
- Immunoglobulin lambda variable 3-9
- Immunoglobulin lambda variable 3-1
- Immunoglobulin lambda variable 3-25
- Immunoglobulin lambda variable 3-12
- Immunoglobulin kappa light chain
- Immunoglobulin kappa variable 1D-16
- Leukocyte-associated immunoglobulin-like receptor 2
CIRS Panel Genes

- CD177 (up 2.14 fold in patients)
- In different clinical conditions, such as myeloproliferative disorder, essential thrombocytopenia, and after granulocyte colony-stimulating factor administration, CD177 becomes significantly up-regulated on the neutrophil surface
CIRS Panel Genes

- Peptidyl arginine deiminase, type VI (up 1.35 fold in patients)
- This enzyme acts post-translationally to convert arginine into citrulline.
- Impacts on the structure and function of the target proteins
- A process thought to occur in several autoimmunity, including rheumatoid arthritis and multiple sclerosis.
CIRS Panel Genes

• Interferon regulatory factor 5 (1.4 fold down regulated in patients)
• IRF5 influences the expression type I interferon and pro-inflammatory cytokine induction
• Has been associated with inflammatory diseases including RA and SLE
Gene Expression in Whole Blood

- Each time point is normalized to the mean
- Tracking genes over time – linking to symptoms

Hemorrhagic rash at this time point
von Willebrand factors

ADAMTS13
aka vWF cleaving protease

Transcript variant 1

Transcript variant 2

vWF domain gene probes
CD86 is an immunoglobulin expressed on antigen-presenting cells that provides co-stimulatory signals necessary for T cell activation.
T regs are dominant in Lyme

• Prevent systemic and tissue based inflammation and auto-immunity
• Thymus and induced
  – TH1 and Th-17; think thymus
  – Mucosal surface (GI and lung); induced
  – Change in microbial communities (induced)
• FOXP3 is transcription factor
More complicated…

- Rising TGF beta-1 sends T regs to the rescue
- Except for plasticity; more TGF beta-1
  - Acquired
  - Developmental FoxP3 (+) to negative
  - Gene regulation (bet there is a microRNA active here)
- Cross-regulatory (IL-10, IL-35, cathepsin E, TRAIL and a lot more)
Big deal in Lyme

- Controls severity of arthritis
- Mediated by IL-17; more IL-17 less destructive arthritis
- With anti-CD25+ Ab, joint destruction induced; delayed anti-CD25 reduces subsequent arthritis
- Passive transfer of CD25+ protects from development of litter mate mice
If low CD25++

- Enhanced pro-inflammatory response to LPS
- But CD4+CD25++ mature in affected joint too; though FOXP3 is scarce in synovial fluid (need more!)
- After antibiotics, synovial fluid receptor for T regs, chemokines and cytokines
Low T regs in joint and CSF

- Antibiotic refractory arthritis lower than antibiotic responsive
- Refractory group also markedly increased FoxP3 (-) compared to (+)
- Same observation in CSF!
T regs

- Low in Post Lyme
- Mechanism: plasticity
- TGF beta-1 directs T regs into tissue to suppress inflammation, including autoimmunity
- If ROR present, no conversion
- If not: disaster, TGF beta-1 made
### T reg changes in Lyme by stage

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Base</th>
<th>Post Abx</th>
<th>Post CIRS</th>
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<tr>
<td><strong>N=</strong></td>
<td>13</td>
<td>34</td>
<td>29</td>
<td>31</td>
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<tr>
<td>TGFB</td>
<td>3621</td>
<td>6782</td>
<td>8967</td>
<td>4890</td>
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<tr>
<td>C4a</td>
<td>3886</td>
<td>8149</td>
<td>6710</td>
<td>4120</td>
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<tr>
<td>C3a</td>
<td>124</td>
<td>1284</td>
<td>384</td>
<td>410</td>
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<tr>
<td>i-Treg</td>
<td>4.66</td>
<td>2.94</td>
<td>3.02</td>
<td>4.16</td>
</tr>
<tr>
<td>t-Treg</td>
<td>4.25</td>
<td>2.44</td>
<td>2.98</td>
<td>3.86</td>
</tr>
</tbody>
</table>
Nasal cultures

- API-STAPH
- Biofilm-formers; slow growing
- Extracellular products
- Genomically active
- RX takes a month
- Reservoir in damp buildings and noses of dogs
Labs to use

- Nasal culture: Diagnostic Lab Medicine, Bedford, Mass
- T regs: Chantilly (Quest affiliated)
- VIP, C4a, C3a Quest only
- TGF beta-1 (Cambridge Biomedical)
- Genomics RUO as of now
  - Physician inquiries only
In case we forget-1

- IL-1B, 4, 6, 6-R, 7, 8, 10,12,15,17, 23
- Chemokines CXCL 1,2,3,4, 5, 10, 13
- HLA 11-3-52B, 15-6-51, 4-3-53
- Toll 1, 2, 4, 5; NFkB; TNF; IFNγ, NK
- MMP1, 3, 9, 13, 14, 19; PAI-1, Osp C
- C3a, C4a, H; NOD2; LPS; Th17 cells
- CD 11c, 14; MASP2; GM-CSF;
In case we forget-2

- Endothelial growth factor
- ACLA; anti-neural AB;
- Caspase-1 (lots to talk about soon)
- Fibronectin, adrenomedullin

- This was just a short list
Treatment steps

VIP

TGF beta-1
- Correct C4a
- Correct C3a
- Correct VEGF
- Correct MMP9
- Correct ADH/osmolality

Correct androgens
- Correct antigliadin
- Eradicate MARCoNS
- CSM/Welchol
- Remove from exposure
Table 4b. Distribution of Case Definition Parameters

<table>
<thead>
<tr>
<th>Number of parameters met by each test subject</th>
<th>CC Cases</th>
<th>Controls</th>
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<tbody>
<tr>
<td>1</td>
<td></td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>15</td>
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<tr>
<td>3</td>
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<tr>
<td>4</td>
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<td>25</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>
CIRS-1

- Once you see it once your life as a physician will be changed forever
- Lack of regulation of inflammation
- Enhanced innate inflammatory parameters (C4a, TGF beta-1, MMP9 and more)
- Hormonal dysregulation
- Hypoxia from capillary hypoperfusion
- And now T regs too
CIRS-2

• Colonizing commensal MARCoNS
• Von Willebrand’s factor-66% abnormal: Acute reactants? NO
• Autoimmunity like crazy! AGA, ACLA, ANA, ANCA, actin
• Cellular immunity: TGF beta-1
• Activated complement split products (C3a, C4a)
VEGF

- Vascular endothelial growth factor
- Responsive to hypoxia inducible factor; feedback from TGF beta-1
- Increases O2 and increases new blood vessel formation
- Judah Folkman, fumagillin and anti-angiogenesis; all about VEGF
Blockade of VEGF a big deal in chemotherapy now; most effective at VEGF (+) receptor tumors

But low VEGF common in the worst biotoxin people (= high before Rx)

Sure some U-shaped skew but low VEGF means cell-based starvation
Capillary hypoperfusion

- Bottom line is decreased delivery of nutrients and oxygen into capillaries
- ABG won’t help; I don’t see where venous gases have academic basis in these illnesses
- Use VO2 max from PST
- Use lactate in MR spectroscopy
VO2 max

- Disability uses this measure a lot
- Look for over 35 in healthy younger person; nomograms available
- 12 ml/kg/min is stage 4 CHF
- So many have VO2 max < 20
- Conversely training to raise VO2 max that doesn’t go beyond anaerobic threshold works in biotoxin people
Raising VO2 max shows benefit

- Correcting VEGF must happen
- Anaerobic threshold is measured
- At exercises start low, go slow
- Defined exercise EVERY DAY
  - Enhanced adiponectin mRNA and protein
- Bike, treadmill; work up to 15’
- Add floor; build up to 15’; then free
- Go back to first defined work, increase sequentially
Post-exertional malaise

- Measure VO2 max in pulm stress test
- It will be low
- What about glycogen in exercise
  - Remarkably inefficient glucose burn
- No O2; no efficiency
  - Can’t say this is mitochondrial illness!
- Fat storage (look at leptin)
- Protein burning (alanine and glutamine)
TGF beta-1

- Dual effects depending on ROR
- Here is the key advancement in assessment of inflammatory illness
- Lung symptoms? Ask re TGF beta-1
- Neuro problems? Ask re TGF beta-1
- Autoimmune? Ask re TGF beta-1
- Learning disability? MS? TM? Same
TGF beta-2

- First found to have increased tissue effect in those with mutant fibrillin-1
- Then the switch to plasma measures
- Normal is < 2380; over 5000 I worry
- Over 10,000 essentially guaranteed restrictive lung disease, tremor, cognitive issues and joint problems
- BBB effects/ gene activation in CNS
TGF beta-3

- Must be double spun plasma
- Platelet contamination common
- If result over 40,000, not properly handled
- Always have second specimen saved
- Cambridge runs the assay
- LC and Quest will forward (Fla**)
MR spectroscopy

- 3 Tesla coil; single voxel
- Frontal lobes and hippocampi
- Same spots! Measure same compounds
- High lactate (> 1.29) too high
- Ratio of glutamate to glutamine (G/G) < 2.19 too low
MRS-2

• Change in cognition is a tip-off
• Reversal of high lactate reverses suppressed G/G
• And Voila! Reversal of cognitive too
• Key concept is that the cellular neuronal mechanisms *are not permanently injured*
VIP-1

- Vasoactive intestinal polypeptide
- Neuroregulatory
- Agonist in suprachiasmatic nucleus
  - Primarily olfactory!
- Binds to membrane receptors
- Activates cellular regulation
- Downregulates cytokines (MMP9)
VIP-2

- Downregulates MASP2
- Restores balance of Vitamin D3
- Downregulates aromatase
- Up-regulates VEGF
- Warning re lipase
- Main effect immediately is endorphin
- Followed by lowering PASP in exercise
PASP and VIP

• 50 mcg QID corrects paradoxical rise in PASP in exercise in days, not weeks, with durable effects with titration to BID and over time: off!

• So many people aren’t diagnosed with acquired PASP even if they have stress echo: must measure TR!

• Don’t accept “normal”
Looks like asthma, but isn’t

- Measure PASP in exercise
- Should not rise more than 8 mm Hg
- Source of palpitations and SOB
- Won’t get better with beta-2 agonists
- Don’t forget EMT and TGF beta-1
- Remodeling in heart, CNS, liver
- Fibrotic change
VIP-3

- Immunoregulatory aspects
- Drives up acquired and thymus derived T regs (CD4+CD25++)
- Here is link from neuropeptides to humoral factors to T-cell physiology
- Role of downregulation of TGF beta-1 has no obvious upper limit in its application
VIP-4

- What doesn’t happen if VIP used inappropriately:
  - Symptoms minimal fall
  - Labs minimal improvement
  - T regs no change
- Must have ERMI< 2;
- HERTSMI-2 < 10
- VCS normal
- Nasal culture by API-staph neg
Long held assumptions: good bye

- New technology in immunology
- 35-parameter mass cytometry
  - 30,000 phenotypes of NK cells
- Germ line versus somatic recomb; variable expression of receptors
- Mutagenesis
- And micro RNA!
  - Nature Immunology 2014; 15(2): 111. editorial
What you need to know

• Symptoms must be there
• Labs must be there to show what is
  – And labs must show what is not
• Differential diagnosis must be there
• The labs will show you the way
  – Start looking at innate immunity as a target
  – Start looking at targets that you can fix
  – Fix the targets; watch the illness disappear
  – Wait for relapse
Here’s my treatment message

- Look for environmental exposures
- Establish a decent baseline of results of innate immunity testing
- Look for biofilm formers; they must go!
- Treat the inflammatory physiology
- What do you have left?
- What happens when the injured patient is exposed next week? Repeat illness!
For more information:

www.survivingmold.com
www.chronicneurotoxins.com

Surviving Mold  December, 2010
Lose the Weight You Hate  2002, 2005