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Unravelling the Origin of Myalgic Encephalomyelitis: Gastrointestinal Dysfunction, Production of Neurotoxins and Environmental Exposure

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Myalgic encephalomyelitis: A highly prevalent debilitating disease

• Persistent, debilitating fatigue associated with numerous physical and neurocognitive symptoms

Disease severity can range from moderate to extremely severe: patients bedridden for years, totally caregiver dependent

• Prevalence estimates: 0.3 to 0.6%; one million patients in the USA, two million patients in Europe

This may just be the tip of the iceberg

• High socio-economic cost

Cost to the society estimated as approximately $16 billion in the USA, €20 billion in Europe
Intestinal disorders in ME patients

- Patients usually present with multiple intestinal symptoms including:
  - Nausea
  - Abdominal pain
  - Poor appetite
  - Abnormal bowel motility
  - Gastric reflux
  - Bloating

- Inflammation of the gastrointestinal tract

- Marked alteration of the intestinal microbial flora
Enterococcus and Streptococcus species are strongly over-represented in ME patients:

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Control</th>
<th>ME patients</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>1.0 x 10^8</td>
<td>4.26 x 10^7</td>
<td>p=0.98</td>
</tr>
<tr>
<td><em>Enterococcus</em> spp.</td>
<td>5.0 x 10^6</td>
<td>3.5 x 10^7</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Streptococcus</em> spp.</td>
<td>8.9 x 10^4</td>
<td>9.8 x 10^7</td>
<td>p&lt;0.001</td>
</tr>
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Among anaerobic bacteria, *Prevotella* is the most consistently overgrown bacteria:

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<tr>
<td><em>Bacteroides</em> spp.</td>
<td>$3.2 \times 10^{11}$</td>
<td>$1.6 \times 10^{11}$</td>
<td>$p=0.39$</td>
</tr>
<tr>
<td><em>Prevotella</em> spp.</td>
<td>$1.0 \times 10^{8}$</td>
<td>$9.0 \times 10^{9}$</td>
<td>$p&lt;0.001$</td>
</tr>
<tr>
<td><em>Bifidobacterium</em> spp.</td>
<td>$6.0 \times 10^{8}$</td>
<td>$5.5 \times 10^{9}$</td>
<td>$p=0.001$</td>
</tr>
<tr>
<td><em>Lactobacillus</em> spp.</td>
<td>$2.7 \times 10^{7}$</td>
<td>$1.8 \times 10^{8}$</td>
<td>$p=0.002$</td>
</tr>
</tbody>
</table>
Bacterial overgrowth correlates with symptoms severity

- *Enterococcus* spp. counts correlate with symptom expression:

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>r and p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>r=0.17, ( p&lt;0.01 )</td>
</tr>
<tr>
<td>Arm pain</td>
<td>r=0.20, ( p&lt;0.003 )</td>
</tr>
<tr>
<td>Shoulder pain</td>
<td>r=0.15, ( p&lt;0.04 )</td>
</tr>
<tr>
<td>Myalgia</td>
<td>r=0.20, ( p&lt;0.003 )</td>
</tr>
<tr>
<td>Palpitations</td>
<td>r=0.16, ( p&lt;0.02 )</td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>r=0.20, ( p&lt;0.004 )</td>
</tr>
</tbody>
</table>
Bacterial overgrowth correlates with symptoms severity

- *Streptococcus* spp. counts correlate with symptom expression:

<table>
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<th>Symptoms</th>
<th>r and p-values</th>
</tr>
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<tbody>
<tr>
<td>Post Exertional fatigue</td>
<td>r=.15, <em>p&lt;0.03</em></td>
</tr>
<tr>
<td>Photophobia</td>
<td>r=.14, <em>p&lt;0.04</em></td>
</tr>
<tr>
<td>Mind going blank</td>
<td>r=.17, <em>p&lt;0.01</em></td>
</tr>
<tr>
<td>Cervical gland lymphodynia</td>
<td>r=.14, <em>p&lt;0.04</em></td>
</tr>
<tr>
<td>Palpitations</td>
<td>r=.15, <em>p&lt;0.03</em></td>
</tr>
<tr>
<td>Dizziness/Faintness</td>
<td>r=.14, <em>p&lt;0.05</em></td>
</tr>
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</table>
Hydrogen sulfide produced by bacteria works as a potent toxin for the body

• Hydrogen sulfide (H$_2$S) has important physiological functions...

H$_2$S is produced by the cells and is an important gaseous signal molecule, involved in regulation of blood pressure, neurotransmission, muscle relaxation and regulation of inflammation

• ...but exogeneous exposure can be extremely toxic

In excess, H$_2$S acts as a mitochondrial poison. It can directly inhibit enzymes involved in the cellular production of energy. H$_2$S also interferes with oxygen transport by blocking hemoglobin in the red blood cells.

Enterococcus, Streptococcus, Prevotella are strong H$_2$S producers
Cumulative effects of H$_2$S and heavy metals

Other gaseous mediators: NO, CO.

Gut
- Strep
- Enterotoxigenic E. coli
- Mold
- Fungi
- Bacteria
- (metals)

Gut barrier
- H$_2$S
- Other gaseous mediators

Cell
- ATP
- Mitochondria
- P$_R$P$^{PC}$
- P$_R$P$^{DX}$
Heavy metals interfere directly with energy production

Extracellular

\[
\text{O}_2^- + \text{NO}^- + \text{Hg}^{2+} \rightarrow \text{ONOO}^-
\]

Plasmamembrane

\[
\text{Oxidase} \quad \text{Cu}^{2+} \quad \text{Q10}
\]

Intracellular

\[
\text{NADH} \rightarrow \text{Krebs cycle} \rightarrow \text{ATP}
\]

Adapted from James Morré 2006 J Inorg Biochem 100 2140-2149
Genetic and environmental factors contribute to aberrant protein conformation.

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**PrPC**

- Genetic
  - Mutations

- Environmental
  - Heavy Metals

- Acquired
  - PrP^dx

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**PrP^dx**
Abnormal conformation can be transmitted from one cell to another
### Disease severity in ME is associated with different physiological dysfunctions

<table>
<thead>
<tr>
<th></th>
<th>I “Pre-ME”</th>
<th>II Moderate disease</th>
<th>III Severe disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dysfunctions</strong></td>
<td>Abnormal faecal test, high H$_2$S</td>
<td>Abnormal faecal test, high H$_2$S, exposure to heavy metals</td>
<td>Abnormal faecal test, high H$_2$S, exposure to heavy metals that has caused aberrant protein conformation (APD)</td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
<td>No fatigue, possible gastro-intestinal symptoms. Low VO$_2$, slow recovery. May be asymptomatic</td>
<td>Fatigue, gastro-intestinal symptoms</td>
<td>Strong fatigue, multiple symptoms</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td>Restore the gut: probiotics</td>
<td>Restore the gut: probiotics, enterocoated antibiotics. Metal chelation, Zinc supplementation</td>
<td>Difficult. Gut restoration, metal chelation. Treatment of associated dysfunctions (opportunistic infections). Treatment of APD is still experimental</td>
</tr>
</tbody>
</table>

Increasing immune dysregulations (depressed T and NK cells, Th17 activation, opportunistic infections….)
Immune alterations resulting from intestinal dysfunction

**Naïve T cells**
- IL-12
- TGF-β + IL-6

**TH1 cells**
- Protection against intracellular pathogens (viruses, bacteria)
- IFN-γ

**TH2 cells**
- Protection against extracellular pathogens (parasites, bacteria)
- IL-4

**TH17 cells**
- Local immunity (mucosa, skin)
- Protection against fungi, bacteria

**Dysbiosis causes a decrease of CD8+ cells and TH1 immunity**

**TH1 downregulation allows increased TH2 and TH17**
Consequences of altered immunity

• T\textsubscript{H}1 decrease favors the development of opportunistic viral infections

HHV-6, Epstein-Barr, parvovirus B19, enteroviruses are found in ME patients. Gastro-intestinal mucosa is a major site of infection

• T\textsubscript{H}2 increase favors the development of allergies

• T\textsubscript{H}17 increase promotes inflammation, autoimmunity, blood-brain barrier disruption

Genetic background plays a role in T\textsubscript{H}17 upregulation

Polymorphisms of IL-17F, IL-6, TLR4, TGF-\(\beta\) genes are associated with ME and other intestinal diseases (Crohn’s disease, UC, IBS)
• Urine test for marker associated with H$_2$S production

• Intestinal microflora evaluation

• Heavy metals analysis

• Presence of proteins with abnormal conformation

• Assays evaluating subsequent immune dysfunctions (immune dysregulations, opportunistic infections...)

A marker associated with H₂S production can be measured with a simple urine test.

1. Collect urine

2. Open tube containing test reagent

3. Add a few drops of urine to the test reagent

4. Mix by shaking gently. Wait for two minutes

5. Observe color changes. Dark color = positive sample

- Negative or Pre-ME
- Moderate disease
- Severe disease
A specific microbiological assay can determine gut microflora populations

- Investigation of the microbial flora of the intestinal tract
  - Quantifies major aerobic and anaerobic bacterial groups and yeast
  - Focuses on dysbiosis (imbalance of the intestinal ecosystem) rather than digestive analysis to ascertain gut integrity

- Challenge: keep anaerobic bacteria viable for analysis
  - Validated oxygen-free, temperature controlled collection and shipping system
• Patient presents increased **Streptococcus, Enterococcus, and Prevotella**
- Patient presents mercury and nickel intoxication
Abnormal protein conformation assay

- Aberrant luminescence response indicates abnormal conformation

![Graph showing luminescence over time for different proteins.](image-url)
CONCLUSIONS

• Gastro-intestinal dysfunctions play a central role in the pathogenesis of ME

• Dysbiosis detrimental effect mediated by increased production of H₂S

• Immune dysfunctions and opportunistic infections arise as a consequence of pre-existing intestinal problems

Once established, infections will contribute to the maintenance/aggravation of the disease
• Henry Butt at the Bio21 Institute, University of Melbourne

• Marian Dix Lemle, Independent Researcher, Washington DC