ALTED IMMUNE FUNCTION IN GWI AND POTENTIAL THERAPIES

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University of Alberta
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Mike Antoni, PhD  CBT/Stress response
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MODEL OF PATHOGENESIS

Genetic Predisposition →

Triggering event / infection →

Mediators (Immune, endocrine, neuroendocrine, sleep, psychosocial, viral reactivation or persistence) →

CFS/ME

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Examples of Immune Cells with Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucocorticoids</td>
<td>T and B-lymphocytes, neutrophils, monocytes/macrophages</td>
</tr>
<tr>
<td>Substance P</td>
<td>T and B-lymphocytes, eosinophils, mast cells, monocytes/macrophages</td>
</tr>
<tr>
<td>Neuropeptide Y</td>
<td>T-lymphocytes, monocytes/macrophages</td>
</tr>
<tr>
<td>Corticotropin Releasing Hormone</td>
<td>T and B-lymphocytes, mast cells, monocytes/macrophages</td>
</tr>
<tr>
<td>Prolactin</td>
<td>T and B-lymphocytes, granulocytes, precursor cells, monocytes/macrophages</td>
</tr>
<tr>
<td>Growth Hormone</td>
<td>T and B-lymphocytes, monocytes/macrophages</td>
</tr>
<tr>
<td>Catecholamines (epinephrine/norepinephrine)</td>
<td>T and B-lymphocytes, neutrophils, NK cells, monocytes/macrophages</td>
</tr>
<tr>
<td>Serotonin</td>
<td>T and B-lymphocytes, NK cells, monocytes/macrophages</td>
</tr>
</tbody>
</table>

The interaction between mediators is key

We approach CFS as an illness with a homeostasis “reset” the new homeostasis set point maintains disruptions of immune, endocrine and autonomic interaction

The immune, autonomic, and endocrine systems find a new balance and promote symptoms

Chronic immune activation is a key component of this model.

Immune abnormalities in GWI

<table>
<thead>
<tr>
<th>Immune Activation</th>
<th>Functional defects</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR, CD26, CD 38 expression</td>
<td>NK Cell dysfunction</td>
</tr>
<tr>
<td>TH2 cytokine shift</td>
<td>CD8 abnormalities</td>
</tr>
<tr>
<td>Proinflammatory cytokines expression</td>
<td>↓perforins, granzymes</td>
</tr>
<tr>
<td>TNF-a, IL-1, IL-6</td>
<td>Macrophage abnormalities</td>
</tr>
<tr>
<td></td>
<td>Antibody production</td>
</tr>
</tbody>
</table>
NK cells and cytotoxic T cells function in a similar way – recognizing a cell, attaching, delivering cytolytic enzymes, releasing and moving to a new target.

The difference is in cell recognition: the more primitive NK cells look for “non-self”; Cytotoxic T cells clone and create large numbers of antigen specific cells.

They are both functioning poorly in GWI

**Perforin** is a molecule in cytotoxic lymphocytes necessary for killing of virus infected and tumor cells.

**Intracellular Cytolytic Granules:**
- Perforin
- Granzyme A
- Granzyme B

**Cell Surface Antigen:**
- CD56
Change in perforin levels during an exercise challenge time series adjusted for the number of NK and CD8+ cells.

The top graph shows intracellular perforin molecules in both NK and CD8 T-cells and the bottom graph the gene expression data (mean signal intensity).

BMC Med Genomics. 2009; 2: 12. Published online 2009 March 5. Impaired immune function in Gulf War Illness Toni Whistler, Mary Ann Fletcher, William Lonergan, Xiao-R Zeng, Jin-Mann Lin, Arthur LaPerriere, Suzanne D Vernon, Nancy G Klimas
Genes regulating NK cell function map differentially in controls and GWI.

The GWI (b) still regulate through TNF and INF-γ pathways, but are also influenced strongly by regulators of apoptosis cell movement and cell adhesion.

Genes regulating perforin and granzymes are also differentially expressed.
CD26 (dipeptidyl peptidase IV) is involved in the activation of T cells, and is expressed on antigen-reactive memory T cells. As reported by our research group, the percentage and number of CD26+ lymphocytes is elevated in CFS and GWI. Quantification of CD26 per cell is reduced in both conditions.

Neuropeptide-Y (NPY) helps to regulate a large number of physiological and pathophysiological processes.

cardiorespiratory system
immune system
nervous system
endocrine system
### Signal molecules Plasma levels

<table>
<thead>
<tr>
<th>Signal molecules</th>
<th>Ctrl</th>
<th>GWI</th>
<th>EGWI - Ectrl</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>12.60 (0.76)</td>
<td>3.45 (0.23)</td>
<td>9.15</td>
<td>0.00</td>
</tr>
<tr>
<td>IL-10</td>
<td>10.07 (0.85)</td>
<td>3.78 (0.19)</td>
<td>6.29</td>
<td>0.00</td>
</tr>
<tr>
<td>TNF-a</td>
<td>0.05 (0.00)</td>
<td>4.89 (0.19)</td>
<td>4.84</td>
<td>0.00</td>
</tr>
<tr>
<td>SCD26</td>
<td>2.13 (0.08)</td>
<td>6.94 (0.53)</td>
<td>4.81</td>
<td>0.00</td>
</tr>
<tr>
<td>NPY</td>
<td>7.71 (0.55)</td>
<td>12.23 (0.84)</td>
<td>4.52</td>
<td>0.00</td>
</tr>
</tbody>
</table>

### PHA-stimulated blood culture

<table>
<thead>
<tr>
<th>PHA-stimulated blood culture</th>
<th>Ctrl</th>
<th>GWI</th>
<th>EGWI - Ectrl</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saliva Cortisol</td>
<td>3.12 (0.15)</td>
<td>6.10 (0.32)</td>
<td>2.98</td>
<td>0.00</td>
</tr>
<tr>
<td>IL-1a</td>
<td>2.12 (0.12)</td>
<td>22.01 (1.68)</td>
<td>19.89</td>
<td>0.00</td>
</tr>
<tr>
<td>IL-5</td>
<td>3.39 (0.28)</td>
<td>13.06 (0.45)</td>
<td>9.67</td>
<td>0.00</td>
</tr>
<tr>
<td>IL-6</td>
<td>2.19 (0.18)</td>
<td>7.09 (0.46)</td>
<td>4.90</td>
<td>0.00</td>
</tr>
<tr>
<td>IL-10</td>
<td>11.75 (0.97)</td>
<td>6.88 (0.62)</td>
<td>4.87</td>
<td>0.00</td>
</tr>
<tr>
<td>TNF-a</td>
<td>2.00 (0.15)</td>
<td>10.23 (0.43)</td>
<td>8.23</td>
<td>0.00</td>
</tr>
<tr>
<td>IFN-g</td>
<td>10.16 (0.44)</td>
<td>10.33 (0.88)</td>
<td>0.16</td>
<td>0.76</td>
</tr>
</tbody>
</table>
The immune abnormalities seen in GWI include immune activation, poor cytotoxic cell function, cytokine regulatory disruptions and abnormalities of neuropeptide Y and cytokines that interface with autonomic, endocrine, and neurologic mediators.

Many of the mediators seen are strong enough to be considered as biomarkers for GWI.

Further, immune activation, pro-inflammatory cytokines and factors that promote this steady state of activation and inflammation are reasonable targets for intervention.

**Conclusion**

- The immune abnormalities seen in GWI include immune activation, poor cytotoxic cell function, cytokine regulatory disruptions and abnormalities of neuropeptide Y and cytokines that interface with autonomic, endocrine, and neurologic mediators.
- Many of the mediators seen are strong enough to be considered as biomarkers for GWI.
- Further, immune activation, pro-inflammatory cytokines and factors that promote this steady state of activation and inflammation are reasonable targets for intervention.
Regulating Protein Expression

- DNA
- RNA
- Protein
- Gene Expression (Microarray)
- RNA sequences
- Transcription
- Translation
- Methylation
- Micro RNA (silencing)
- Biological Function
- Genome sequences

Micro RNA (silencing)

Regulating Protein Expression
Complete the data set needed for the comprehensive systems biology analysis of GWI

Underway: DOD study with 8 assessment points post exercise to better map out the systems interactions

Pending review (Merit): Methylation/microRNA analysis of the same cohorts

LOI accepted (DOD): Proteomic/metabolomics study

CSP 585 assessing genetic risk
Apply computational models to develop intervention strategies to GWI
In development: DOD Consortium protocol focused on systems biology/computational modeling in animal and human systems to develop additional models
LOI accepted: Phase 1 trial of IL1 receptor antagonist in GWI
Propagation through Time of CD2+/CD26+

Healthy Controls

GWI Patients

Sub-optimal Approximation of $u(t)$

- Treatment amplitude or intensity
- Treatment duration

**More realistic or practical implementation of numerically optimal treatment**

Optimal Manipulation of HPA Axis

- How low and how long:
  - Could administer treatment in stages: looking for a 20% increase in ACTH at rest.
  - Smaller reductions in cortisol would require longer treatment.
  - There is a minimal perturbation however below which the system will simply return to its fatigued state.
Time for questions, but first thank you!