From Cytokines to Cells to Gene Expression: An Integrative Approach to the Study of Gulf War Illness

Gordon Broderick, Ph.D.
Associate Professor, Dept. of Medicine,
University of Alberta

Nancy Klimas, M.D., Miami Veterans Affairs Medical Center
Mary Ann Fletcher, Ph.D., University of Miami
Sol Efroni, Ph.D., Bar Ilan University

Systems Biology to Systems Medicine

- Key: Integration and context
- Integration and reconciliation of types of data
- Integration of components within /across functional levels.
- Why? Context-sensitive environment

Not Just a Collection of Parts

Tightly integrated and highly interactive components

Brain/ Nervous System
Immune System
Endocrine (hormone) System

Stable Efficient Energy Utilization

Basic premise: Illnesses will differ not only in the expression of markers but in their patterns of association or interaction.

Comparing Two Networks

Compare based on changes needed to transform one into the other… Can do this at multiple scales
Comparing several networks

- Cytokine networks differ significantly in distribution and strength of associations $D_{edit} = 1.96$
- $>10$ std dev. over separation of networks from randomly sampled HC subjects ($D_{edit} \approx 0.18$)

Describing Basic Network Structure

Others include path-dependent measures such as:
- Average path length, network diameter, etc…
- Edge betweenness centrality, modularity index, etc…
A Scalable Design in Biology

Facebook or immune networks\(^1\): scale free *hub-centric* networks are ubiquitous across scales in biology...


A Granular Composition

Modularity with number of sub-networks controls (red) and CFS (blue).

- Significant differences at aggregation thresholds: N=5 and 13 cliques.
- CFS shows very early separation of immune nodes i.e. N=5 cliques

Altered Immune Communication

- Immune network wiring looks different; altered immune homeostasis.
- Highly attenuated Th1 and Th17 responses.
- High Th2 expression and interactions pointed to allergic inflammation.
- Indirect evidence of diminished NK cell responsiveness to IL-12 and Lta.

Cohort of adult female CFS patients


GWI Study

Participants
- Comparing veterans with GWI to veterans with CFS
- Using standard Graded Exercise Test (GXT) to question the system
- Initial set of n=10 GWI and 11 healthy veterans

Measuring:
- Immune signaling proteins, stress hormones, immune cell populations and gene expression in immune cells
- Pre-exercise, peak effort (max VO2), and 4 hours post-exercise
Bringing It Together: An Immune Response Network

1(a). Ctrl at rest (t0)

1(b). GWI at rest (t0)

Edit D(t1) = 3.89 (0.036); 44 pooled std error

1(c). Ctrl at peak effort (t1)

1(d). GWI at peak effort (t1)

Edit D(t2) = 4.65 (0.038); 60 pooled std error
Bringing It Together: An Immune Response Network

1(e). Ctrl 4hrs post-exercise (t2)

Edit D(t3) =3.75 (0.046); 42 pooled std error

1(f). GWI 4 hrs post-exercise (t2)

GWI: A very Different Immune Response Strategy

GWI: more abundant active connections

HC: Not much change in general architecture

GWI: more diffuse, less organized, fewer hubs
Agents of Change

2(b). GWI - Ctrl at t1
IL-5, sCD26 mounting B cell response with IL-6 Th17?

Altered NPY, TNFα CD26 energy usage?

2(a). GWI - Ctrl at t0

Delayed IL-6 response to exercise (insulin sensitive)

2(c). GWI - Ctrl at t3

Propagation through Time of IL-1α

Post-exercise (t2)

Peak effort (t1)

At rest (t0)

Healthy Controls

GWI Patients

Propagation through Time of CD4+/26+

Healthy Controls

GWI Patients


Looking Within Immune Cells

Attaching to known “matching” messages

Reading each gene’s contribution

Tagging genetic message (mRNA)

cDNA Microarray

Immune cells from blood
Survey of Lymphocyte Gene Expression

- Surveyed whole genome in *duplicate samples* using the Affymetrix GeneChip Human Genome (HG) U133 Plus 2.0 Array *
- All probe data was RMA background-adjusted and quantile normalized using the Affymetrix Power Tools (APT) platform.
- Sample set extended to include:
  - n= 20 GWI subjects
  - n= 7 CFS subjects
  - n= 22 healthy control subjects
- Sampled at rest prior to exercise, at maximum effort and 4 hours post-exercise.

* Dr. Lubov Nathanson; Hussman Institute for Human Genomics, Univ. of Miami.

Lymphocyte Gene Expression at Peak Effort

- 50 genes uniquely *over-expressed* under effort in GWI at FDR<0.05
- 59 genes uniquely *down-expressed* in CFS, none in GWI...
Identifying Active Genes in Individual Patients

• Map to Up/Down discrete states for every gene in each individual sample
• Model expression values to a mixture of gamma distributions;


Mapping Active Genes to Active Pathway Elements

• Gene expression supports directed functional interactions
• Pathway elements in NCI-Nature Pathway Interaction Database (PID)1
• Assess if combination of gene activity levels support rule consistently

1 http://pid.nci.nih.gov/
Differences in Pathway Activity in PI-CFS

- 112 GWS, 90 CFS
  - 585 pathways show group effects with FDR < 0.05

Differences in Metabolic Pathway Activity in CFS

- 36 pathways specific to CFS vs GWS and Ctrl related to cell metabolism
- Purinergic (cy alanine/aspartate), amino, nucleic acid metabolism
## Differences in Signaling Pathway Activity in CFS

- 25 pathways specific to CFS vs GWS and Ctrl related to cell signaling
- Suggest disengagement of innate immune signals and lymphocyte repair

### Toll like receptor signaling (KEGG)

<table>
<thead>
<tr>
<th>Node degree</th>
<th>CFS</th>
<th>Ctrl</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 in CFS, 30 in Ctrl</td>
<td>5 in CFS, 22 in Ctrl</td>
<td></td>
</tr>
</tbody>
</table>

### Rac1 cell motility signaling (Biocarta)

<table>
<thead>
<tr>
<th>Node degree</th>
<th>CFS</th>
<th>Ctrl</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 in CFS, 1 in Ctrl</td>
<td>20 in CFS, 9 in Ctrl</td>
<td></td>
</tr>
</tbody>
</table>

## Differences in Metabolic Pathway Activity in GWS

- 23 pathways specific to GWS vs CFS and Ctrl related to metabolism
- Imbalanced redox pathways - lowered defense against oxidative stress

### Nicotinate/nicotinamide metabolism

### Pentose/glucuronate interconversions (KEGG)

<table>
<thead>
<tr>
<th>Node degree</th>
<th>GWS</th>
<th>Ctrl</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 in GWS, 1 in Ctrl</td>
<td>20 in GWS, 9 in Ctrl</td>
<td></td>
</tr>
</tbody>
</table>
Differences in Immune Signaling Pathway Activity in GWS

• 57 pathways specific to GWS vs CFS and Ctrl related to cell signaling
• Engagement into network of dampened immune signaling (adaptive)

Differences in Immune Signaling Pathway Activity in GWS

• Stable immune hubs heightened in activity but down-modulated by effort
• Broad regulator of immune response chronically active
Ripple Effect in Other Signaling Pathways in GWS

Thromboxane a2 rec signaling (NCI/Nature)  
EGR (NGF) control pathway

Node degree: 11 in GWS, 9 in Ctrl  
Node degree: 1 in GWS, 3 in Ctrl

- Stable hemodynamic hub decreased in activity, opposite to CFS
- Early growth response (EGR) lowered under effort like CFS but more so

Differences in Pathway Activity in GWS and CFS

- First general observations
  - Immune *metabolic function* majority of disrupted pathways in CFS
  - Irregular immune and associated signaling main theme in GWS
  - Different aspects of *alanine-aspartate / phenylalanine metabolism*  
    affected in both CFS and GWS
  - Suppression of *1- and 2-methylnaphthalene degradation* pathway common to both illnesses, similar discriminator.

- Another part of the picture
  - Increased *recruitment* of these pathway segments into larger network in GWS vs disengagement in CFS…
Transforming one TF network into another…

• CFS and GWI TF networks differ significantly from that of controls
• TF network for GWI is more distant from control than CFS

Transforming one TF network into another…

• TF network for GWI is structurally different from the TF network for CFS
• They are more similar to one another than either is to the control TF network
Mapping Active Genes to Active Pathway Elements

Differentially Engaged Known Pathways

- Disengagement of growth factor signaling and tissue repair in ME/CFS
- Opposite true in GWI; distinct porphyrin (heme) and ceramide (apoptosis) signaling (neither is differentially active*)
Mapping Active Genes to Active Pathway Elements

- Ceramide: inflammatory hub among hubs...
- Embedded with immune (B cell receptor, focal adhesion, MapK), repair/growth (PDGF, NGF), broad signaling (angiotensin, GnRH)

GWI Network of pathway elements

**High node degree sub-net**

Synopsis

A. Activity of individual pathway segments
   - Several pathways differ significantly in activity during exercise
   - Majority are unique to either GWS or CFS

B. Interaction of pathways segments
   - Significant differences in patterns of association
   - GWS recruiting new associations
   - CFS shedding normally active associations

C. Flow of regulatory information
   - Ongoing. Requires the construction and analysis of directed graphs.
Accelerating the Design of Treatment Trials

- Early models point to manipulation of bound cortisol for HPA reset


Acknowledgement of Funding

- United States Department of Veterans Affairs
- CONGRESSIONALLY DIRECTED MEDICAL RESEARCH PROGRAMS
- National Institutes of Health
- CFIDS Association of America
- PANODRAM, Inc.