Background

**Gulf War Illness (GWI): multi-system chronic disorder.**
- Unexplained cognitive, musculoskeletal and fatigue symptoms.
- Of ~700,000 Gulf War troops deployed estimated 10% PTSD,
- 2.5% satisfied the criteria for FM
- 3-5% met the modified case definition for CFS¹.

**Evidence supporting some basic components…**
- Immune imbalance: abnormal T cell proliferation, NK cell function…
- Neuroendocrine imbalances: blunted mediation of innate immunity and cortisol

¹ Bourdette et al, J Occup Environ Med. 2001;43:1026–1040
Looking at the Bigger Picture in Wichita

Immunity’s ripple effect on 30 major hormones and neurotransmitters

**Significant Local Remodeling**

**Immune cell-specific gene sets:**
- Altered B cell function
- Monocytes, neutrophils more central

**Free thyroxin T4:**
- Increased immune activity
Mapping Neuroendocrine-immune Interaction

Key findings

• Emergent immune network aligning with known model of persistent inflammation.
• Increased path length between ACTH and cortisol: decoupled HPA?
• Surge in immune interaction with active T4; thyroid autoimmunity?

Open questions…

• Increase survey of immune signals: cytokine and cell population assays
• How do these immune network changes apply to Gulf War Illness?
• What about response dynamics?

Introduction

Hypothesis.

• GWI subjects can be distinguished from healthy veterans (and CFS) by their neuroendocrine-immune status.
• More specifically by modifications to the cell-cell communication networks used to regulate function
• Differences can be amplified by studying response to exercise.

Cohort.

• Subset of 10 GWI and 11 healthy controls recruited from the Miami Veterans Administration Medical Center;
• All male and ranging in age 30-55; matched by age, BMI, ethnicity
• Exclusionary criteria based on Fukuda et al (1998)
Experimental Assessment

Sample collection and analysis.

- Standard Graded eXercise Test (GXT)
- Collection of peripheral blood at 3 points: pre-exercise, at peak effort (max VO\textsubscript{2}), and 4 hours post-exercise
- Assessment of 6 cytokines, soluble CD26, NPY and cortisol
- Repeated 3 cytokines in plasma (IL-6, 10 and TNFa)
- Flow cytometry detailing 11 subsets of T, T-helper, NK, and B lymphocyte populations

Numerical analysis.

- Differences in expression at each time point (pseudo steady state)
- Differences in association patterns:
  - Combinatorial expression: “Grey Box” Linear Discriminant Classifier
  - Network architecture: redistribution and extent of connectivity

• Looking for diagnostic features and illness processes
3 Levels of Resolution

- Intercellular signal (ELISA)
- Cellular response (flow cytometry)
- Intracellular signal (microarray)

Individual Intercellular Signals

<table>
<thead>
<tr>
<th>Signal molecule</th>
<th>GW-01-Ctr(0)</th>
<th>GW-01-Ctr(1)</th>
<th>GW-01-Ctr(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuropeptide Y (NPT)</td>
<td>-16.38 (0.14)</td>
<td>-16.28 (0.54)</td>
<td>-14.38 (0.36)</td>
</tr>
<tr>
<td>Cortisol (Blood)</td>
<td>0.12 (0.21)</td>
<td>0.05 (0.30)</td>
<td>0.00 (0.00)</td>
</tr>
</tbody>
</table>

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Some first observations:
- Significantly higher IL-5 and IFNγ at all 3 conditions; borderline IL-6 in plasma
- Significantly higher TNFα at rest,
- Significantly *higher* cortisol post-exercise but borderline *lower* at peak effort
Cytokines Good Building Blocks for Distinguishing GWI

- A simple classifier could start by using IL-5 and IFNg at a single time point
- What is best time point? IL-5 levels get closer under effort while IFNg levels diverge

Ideally Use Entire Cytokine Response over Time

- Multiple cytokines have a de-noising or averaging effect!
- Using entire time course profile adds additional discrimination
Separating Specific Details from the General Response

- Composite feature 1 is a general upset in all cytokines
- Composite feature 2 is additional coordinated cortisol ↓, IL-1α ↑, IL-10 ↓ and IL-6 ↓

GWI Subjects are Distinct in Immune Response to Exercise

- Feature 1 and 2 are defined by combinations of cytokines
- Each point captures the complete time course for that subject
GWI Subjects are Distinct in Their Cytokine Response to Exercise

- Feature 1 is a general upset in all cytokines
- Higher with transient increase in GWI; lower and slow rise in Controls
- Differences in this general upset are significant throughout the challenge

GWI Subjects are Distinct in Their Cytokine Response to Exercise

- Feature 2 is an additional coordinated cortisol ↓, IL-1α ↑, IL-10 ↓ and IL-6 ↓
- Changes in this pattern are best distinguished post-exercise (T2)
A First Synopsis

- Cytokines used in combination and across time respond to exercise completely separates diagnostic groups.
- Broad spectrum changes (Feature 1): distinguishable throughout challenge but insufficient for optimal separation.
- Changes in co-expression of cortisol, IL-1α, IL-6 and IL-10 (Feature 2) improve separation of subjects and are best distinguishable post-exercise.

Cell Proliferation in the Context of Immune Signaling

- Uniformly depressed NK populations exacerbated by effort,
- Significantly higher CD8+/CD26+, CD2+ at rest and at peak effort; delayed response.
Bringing It Together: An Immune Response Network

1(a). Ctrl at rest (t0)

Cell abundance
- Cortisol
- NK cytotoxicity

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

1(b). GWI at rest (t0)

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

Broderick Laboratory for Computational Medicine
Agents of Change

- Altered NPY, TNFα, CD26 energy usage?
- Delayed IL-6 response to exercise (insulin sensitive)

A Second Synopsis

- GWI networks *bigger and less organized (less efficient?)*
- At rest NPY, IL-1, and TNF-α with CD2+/CD26+ abundance exert broad influence on GWI network.
- Under exercise the GWI network promotes IL-5, sCD26 stimulation of CD19+ B cells
- Exercise induces IL-6-mediated Th2 differentiation and/or Th17 responsiveness.
- Post exercise see delayed plasma IL-6 response suggesting shift in fat regulation and energy metabolism.
A Working Model?

- Increased IL-6, TNFa in plasma support low-grade persistent inflammation.
- High IFNg responsiveness supports active Th1 response (as in most autoimmune)
- High IL-5 responsiveness and increased linking of CD19+, IL-5, sCD26 and Il-6 supports active Th2 response (conventional allergic).
- Foreign antigen mimicking intracellular self known to induce mixed Th1/Th2 response
- i.e. Hom s1, Hom s4 both IgE-reactive and induce IFNg response.

A Working Model?

Exogenous

- Cross-reactive allergens

IgE-mediated allergic tissue inflammation

Endogenous

- Autoimmune tissue damage

Cell damage and release of autoantigens

Cross-reactive T cell activation
A Working Model?

- IL-6 is "exercise cytokine"; flag for low local energy reserves
- Altered IL-6 co-expression with NPY, IL-1, IL-10: blunted insulin sensitization response.
- Soluble CD26 also recruited; inhibits amplification of insulin response
- Elevated TNFα responsiveness promoting insulin resistance.
- Low NPY with high TNFα also seen is anorexia

*Autoimmune inflammatory environment also limiting adequate access to metabolic energy?*

Ongoing work: Propagation through Time

Healthy Controls

GWI Patients
Ongoing Work: Propagation through Time

Propagation through Time of NPY
Propagation through Time of CD2+/26+

- At rest (t0)
- Peak effort (t1)
- Post-exercise (t2)

Healthy Controls

GWI Patients

Propagation through Time of IL-1a

- At rest (t0)
- Peak effort (t1)
- Post-exercise (t2)

Healthy Controls

GWI Patients
Ongoing Work: Comparison of Intracellular Signal

**Feature 1**
- \( R_{probe}^2 = 0.18 \)
- \( R_{class}^2 = 0.69 \)

**Feature 2**
- \( R_{probe}^2 = 0.07 \)
- \( R_{class}^2 = 0.26 \)

**Feature 3**
- \( R_{probe}^2 = 0.07 \)
- \( R_{class}^2 = 0.04 \)

Top 1000 probe set instances: 171 at T0, 612 at T1, 217 at T2
Median signal to noise: 3.97

Comparison of Intracellular Signal

**Feature 1 & 2**
- \( R_{probe}^2 = 0.24 \)
- \( R_{class}^2 = 0.95 \)
- \( Q^2 = 0.52 \)

++ Signal transduction, Cell communication

++ Nucleic acid metabolism / cell cycle
Protein metabolism and modification

Nucleic acid metabolism/ transcription regulation
Peroxisome transport / Oxidative phosphorylation
Protein lipid, fatty acid and steroid metabolism
Interferon-mediated immunity

Probe weight \( t > 2.0 \)
All processes \( p < 0.05 \)
Panther Classification
Comparison of Intracellular Signal

KIFAP3 kinesin-associated protein 3 (probe set 203333_at)

GWI  Ctrl

Gene POP5 (probe set 204839_at)

ABCC2 ATP-binding cassette (probe set 206155_at)

Team Effort

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Helping training the next generation of clinician