
Immune network remodeling in Chronic Fatigue Syndrome and Gulf War Illness

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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

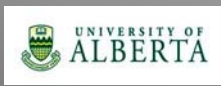
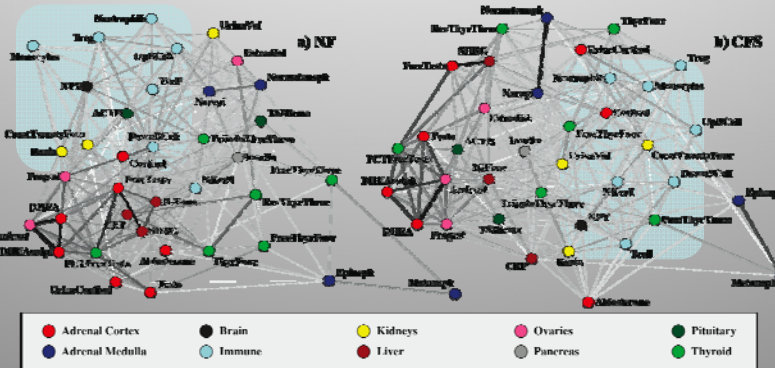
¹ Bourdette et al. *J Occup Environ Med.* 2001;43:1026-1040



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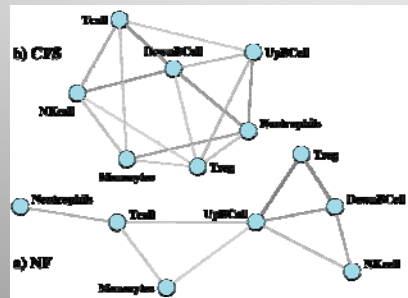
Looking at the Bigger Picture in Wichita

Immunity's ripple effect on 30 major hormones and neurotransmitters

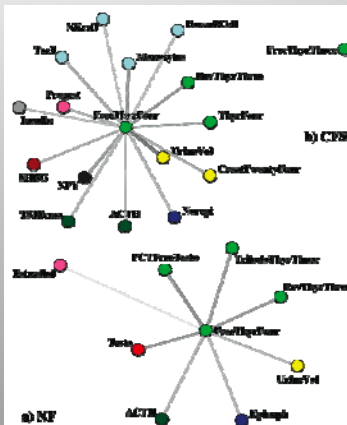


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Significant Local Remodeling



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



- Free thyroxin T4:
- Increased immune activity



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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?



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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)



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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_2), and 4 hours post-exercise
- Assessment of 6 cytokines, soluble CD26, NPY and cortisol
- Repeated 3 cytokines in plasma (IL-6, 10 and TNF α)
- Flow cytometry detailing 11 subsets of T, T-helper, NK, and B lymphocyte populations



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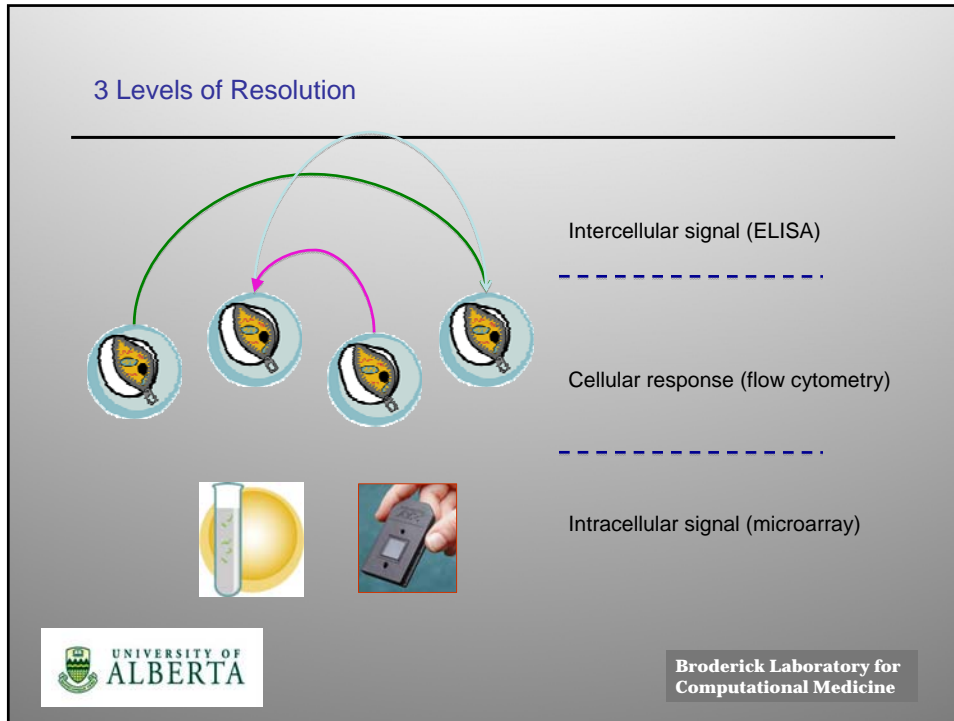
Experimental Assessment

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*



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Individual Intercellular Signals

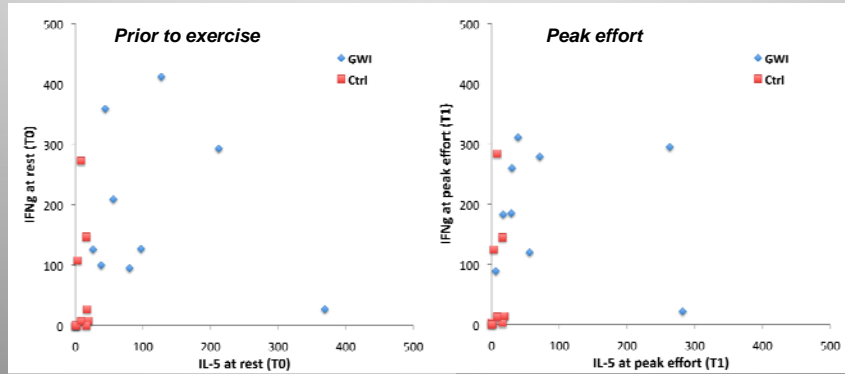
Signal molecule	GW(0) - Cit(0)	GW(1) - Cit(1)	GW(2) - Cit(2)
Neuropeptide Y (NPY)	-28.83 (0.87)	-14.88 (0.34)	-14.30 (0.38)
Cortisol (Salivary)	0.12 (0.21)	-0.08 (0.08)	0.08 (0.03)
<i>ex vivo PHA-stimulated Blood Culture</i>			
IL-1a	2.33 (0.71)	13.00 (0.71)	2.03 (0.78)
IL-5	86.53 (0.00)	38.54 (0.00)	41.00 (0.00)
IL-6	-565.00 (0.88)	1088.00 (0.30)	2875.00 (0.38)
IL-10	21.00 (0.83)	-248.00 (0.14)	-14.80 (0.80)
TNF α	247.02 (0.02)	-25.00 (1.00)	88.89 (0.06)
INF γ	117.71 (0.02)	170.76 (0.02)	157.10 (0.03)
sCD28	-143.00 (0.16)	-20.00 (0.67)	-81.00 (0.73)
<i>In vivo Unstimulated Plasma</i>			
IL-6 (plasma)	10.70 (0.03)	10.30 (0.06)	8.10 (0.06)
IL-10 (plasma)	-1.80 (0.21)	-3.90 (0.31)	-2.20 (0.28)
TNF α (plasma)	2.88 (0.08)	1.16 (0.37)	1.30 (0.25)

Some first observations.

- Significantly higher IL-5 and IFN γ at all 3 conditions; borderline IL-6 in plasma
- Significantly higher TNF α at rest,
- Significantly *higher* cortisol post-exercise but borderline *lower* at peak effort


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Cytokines Good Building Blocks for Distinguishing GWI

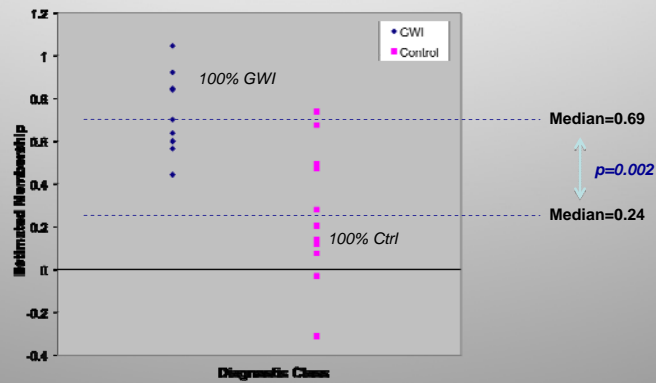


- A simple classifier could start by using IL-5 and IFNγ at a single time point
- What is best time point? *IL-5 levels get closer under effort while IFNγ levels diverge*



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Ideally Use Entire Cytokine Response over Time

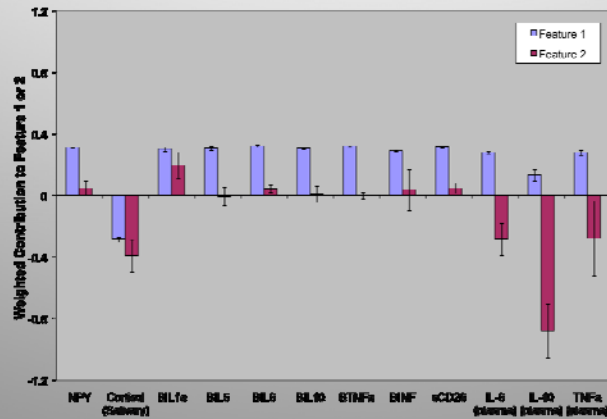


- Multiple cytokines have a *de-noising or averaging effect!*
- Using entire time course profile adds additional discrimination



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Separating Specific Details from the General Response

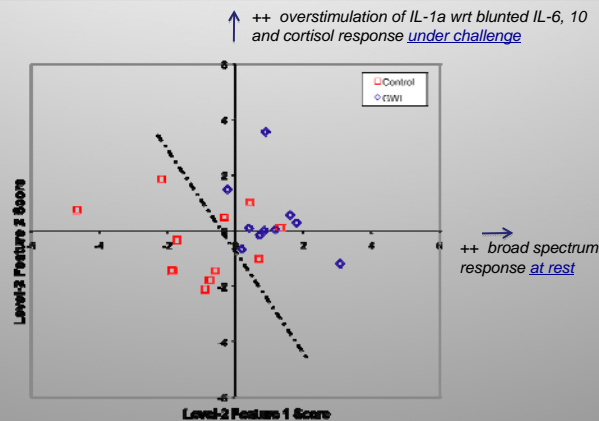


- Composite feature 1 is a general upset in all cytokines
- Composite feature 2 is additional coordinated cortisol ↓, IL-1 α ↑, IL-10 ↓ and IL-6 ↓



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GWV Subjects are Distinct in Immune Response to Exercise

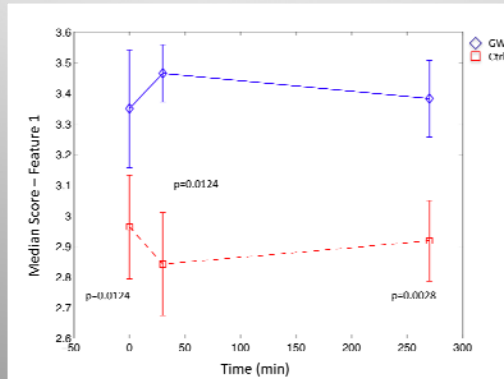


- Feature 1 and 2 are defined by combinations of cytokines
- Each point captures the complete time course for that subject



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GWVI Subjects are Distinct in Their Cytokine Response to Exercise

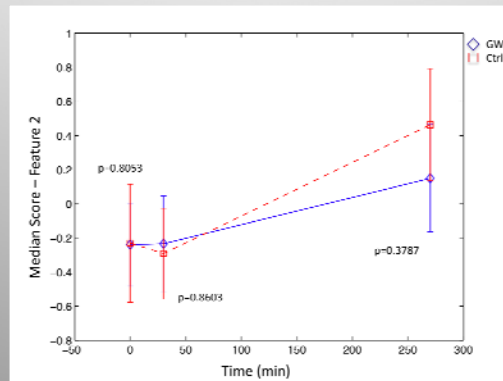


- Feature 1 is a general upset in all cytokines
- Higher with transient increase in GWVI; lower and slow rise in Controls
- Differences in this general upset are *significant throughout the challenge*



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GWVI Subjects are Distinct in Their Cytokine Response to Exercise



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



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A First Synopsis

- Cytokines used in combination and across time *response 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-1 α , IL-6 and IL-10 (Feature 2) improve separation of subjects and are best distinguishable post-exercise



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Cell Proliferation in the Context of Immune Signaling

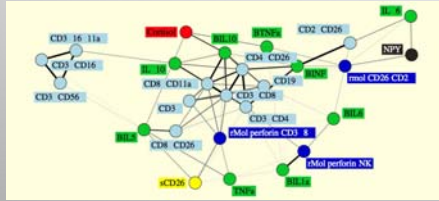
Cell subset	GM(t0) - Ctr(t0)	GM(t1) - Ctr(t1)	GM(t2) - Ctr(t2)
B cells			
CD19+%	-1.72 (0.60)	1.71 (0.26)	-1.95 (0.67)
T lymphocytes			
CD3+CD4+%	-2.70 (0.48)	3.95 (0.78)	-3.83 (0.55)
CD3+CD8+%	10.95 (0.44)	12.53 (0.47)	9.93 (0.44)
CD3+%	5.78 (0.60)	8.83 (0.39)	0.20 (0.60)
NK cells			
CD3-CD56+%	-1.32 (0.03)	-7.28 (0.14)	-5.52 (0.01)
CD3-CD16+%	-2.83 (0.02)	-12.37 (0.04)	-6.70 (0.02)
CD3-16+11a+%	-5.99 (0.01)	-14.71 (0.02)	-6.60 (0.01)
Cytotoxic and helper T cells			
CD2+CD26+%	5.39 (0.06)	13.22 (0.04)	8.62 (0.11)
CD4+CD26+%	4.30 (0.86)	7.05 (0.39)	-0.02 (0.80)
CD8+CD26+%	16.84 (0.02)	20.36 (0.02)	16.75 (0.08)
CD8+CD11a+%	1.48 (1.00)	-1.73 (0.49)	0.88 (1.00)
Cytotoxicity			
molCD26/per CD2+ cell	-300.73 (0.73)	-654.00 (0.22)	152.00 (0.60)
mol perforin/NK cell	700.23 (0.55)	-1250.00 (0.08)	59.32 (0.49)
mol perforin/CD3+CD8+ cell	169.35 (0.73)	-65.01 (0.60)	-282.30 (0.30)

- Uniformly depressed NK populations exacerbated by effort,
- Significantly higher CD8+/CD26+, CD2+ at rest and at peak effort; delayed response



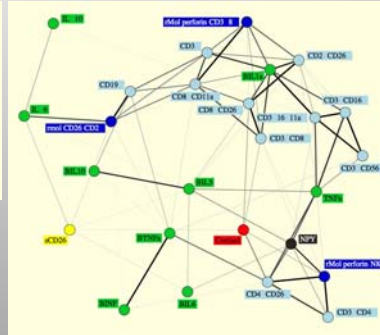
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Bringing It Together: An Immune Response Network



1(a).Ctrl at rest (t0)

- Cell abundance
- Cytokines
- Cortisol
- NK cytotoxicity



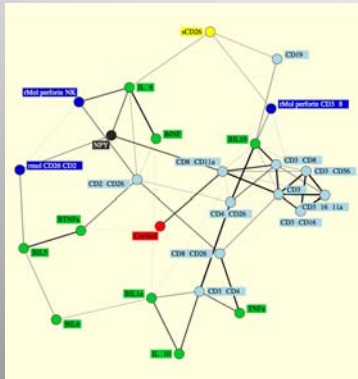
1(b). GWI at rest (t0)

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



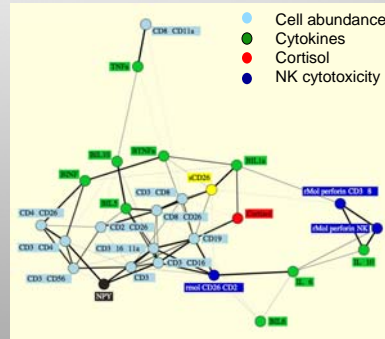
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Bringing It Together: An Immune Response Network



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

Edit D(t2) = 4.65 (0.038); 60 pooled std error

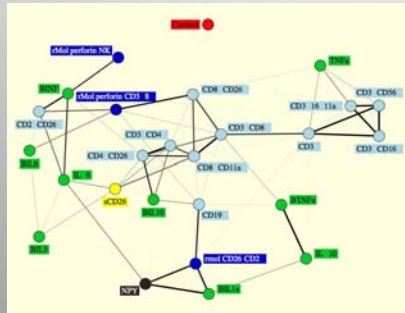


1(d). GWI at peak effort (t1)

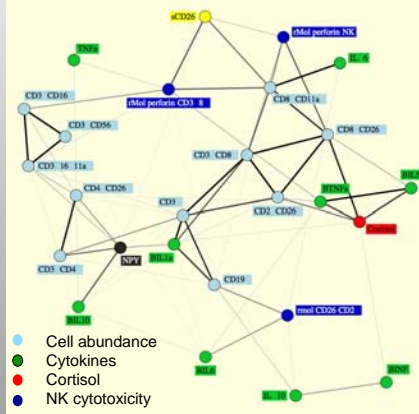


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Bringing It Together: An Immune Response Network



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



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

- Cell abundance
- Cytokines
- Cortisol
- NK cytotoxicity

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

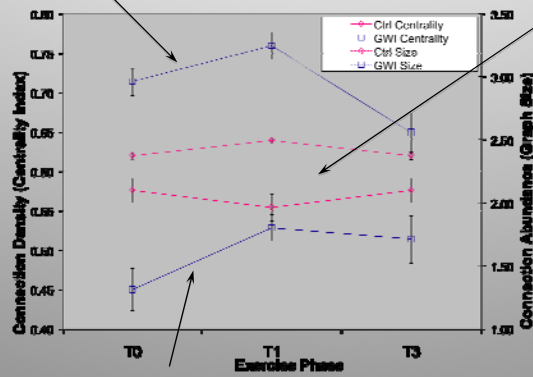


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GWVI: A very Different Immune Response Strategy

GWVI: more abundant active connections

HC: Not much change in general architecture



GWVI: more diffuse, less organized, fewer hubs



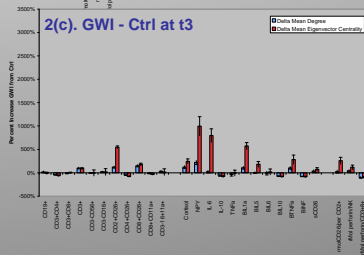
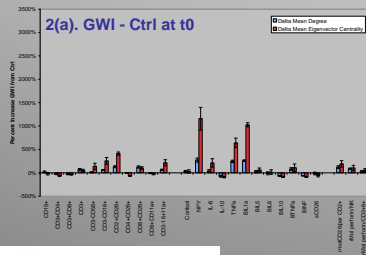
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Agents of Change

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

Altered NPY, TNFa CD26 energy usage?

Delayed IL-6 response to exercise (insulin sensitive)



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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.



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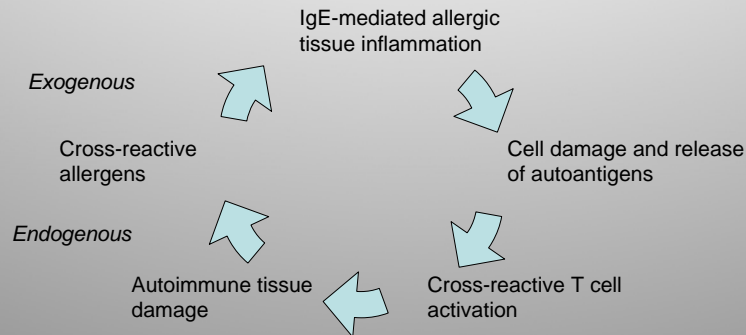
A Working Model?

- Increased IL-6, TNF α in plasma support low-grade persistent inflammation.
- High IFN γ 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 IFN γ response**.



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A Working Model?



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A Working Model?

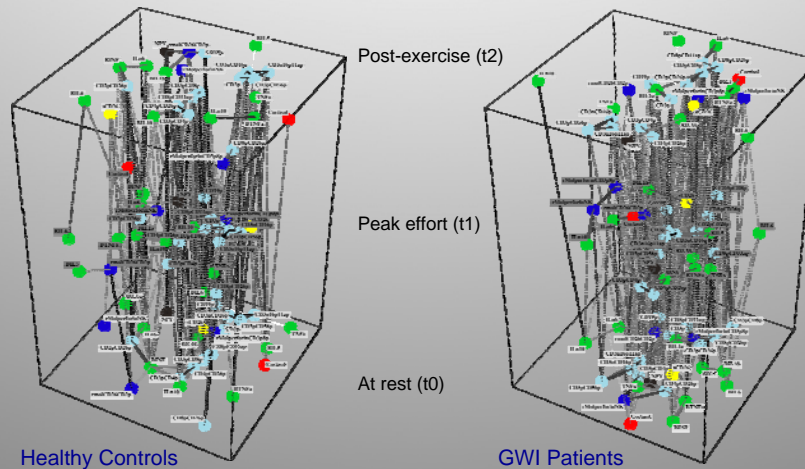
- IL-6 is “*exercise cytokine*”; flag for low local energy reserves
- Altered IL-6 co-expression with NPY, IL-1, IL-10: **blunted insulin sensitization response**.
- Soluble CD26 also recruited; *inhibits* amplification of insulin response
- Elevated TNF α responsiveness promoting *insulin resistance*.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

Autoimmune inflammatory environment also limiting adequate access to metabolic energy?



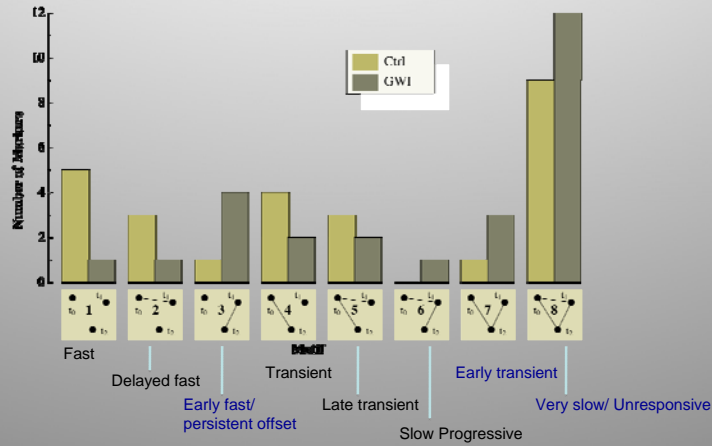
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Ongoing work: Propagation through Time



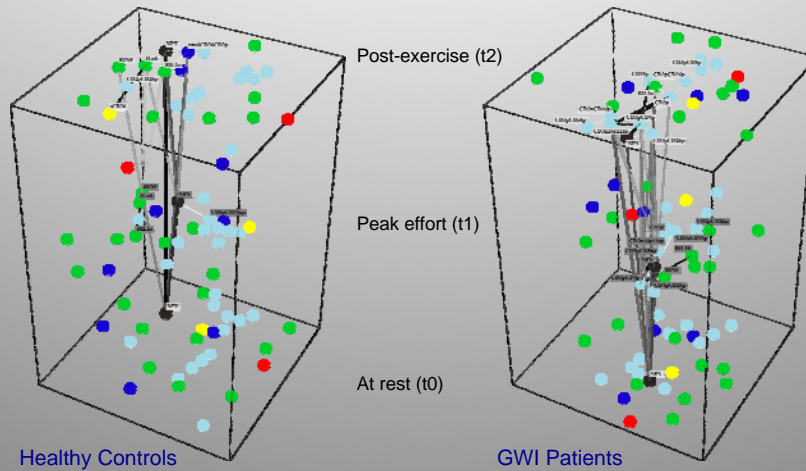
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Ongoing Work: Propagation through Time



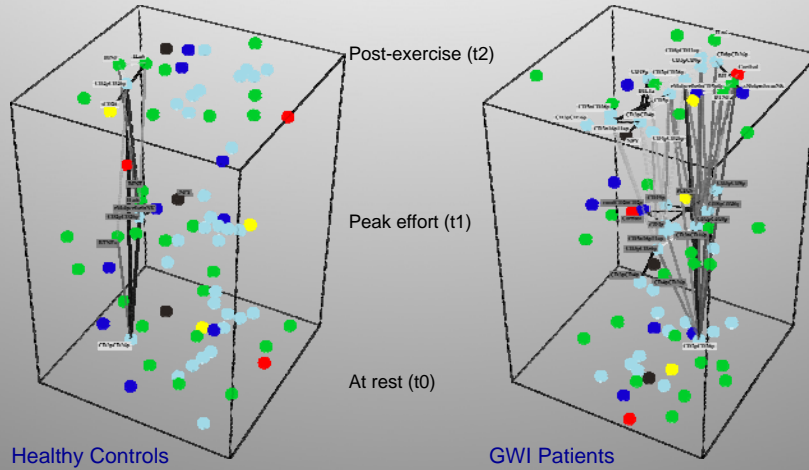
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Propagation through Time of NPY



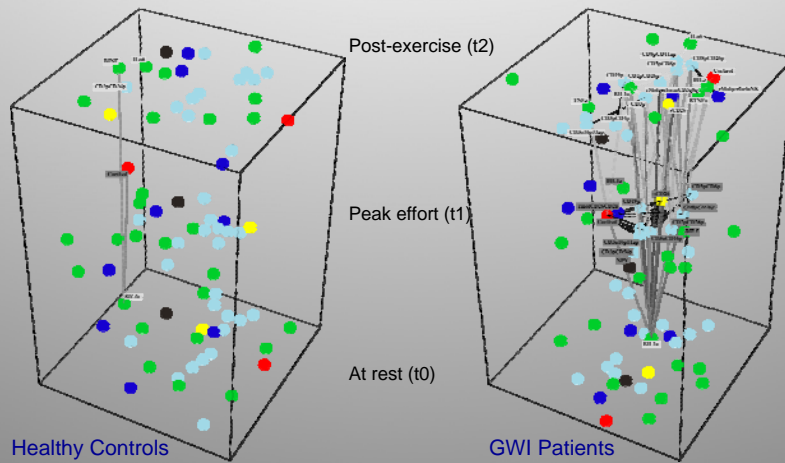
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Propagation through Time of CD2+/ 26^+



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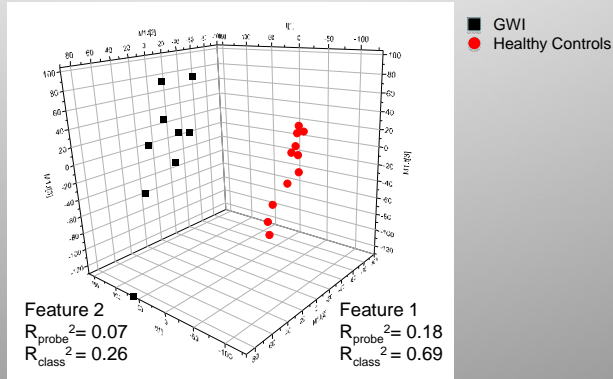
Propagation through Time of IL-1 α



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Ongoing Work: Comparison of Intracellular Signal

Feature 3
 $R_{probe}^2 = 0.07$
 $R_{class}^2 = 0.04$



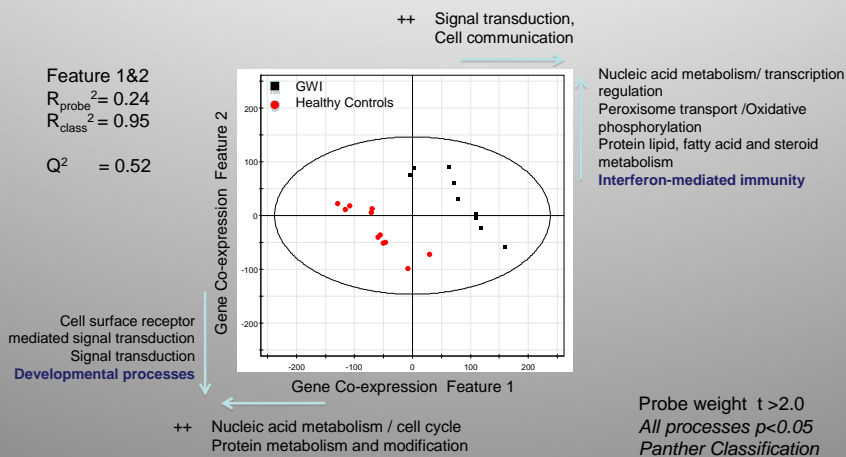
Top 1000 probe set instances: 171 at T0, [612 at T1](#), 217 at T2
 Median signal to noise: 3.97



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Comparison of Intracellular Signal

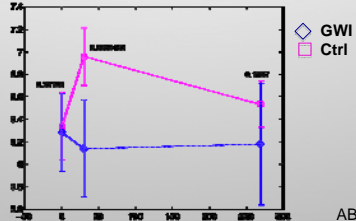
Feature 1&2
 $R_{probe}^2 = 0.24$
 $R_{class}^2 = 0.95$
 $Q^2 = 0.52$



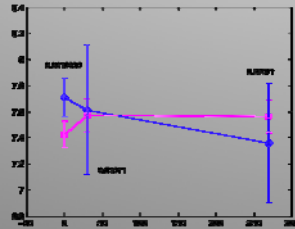
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Comparison of Intracellular Signal

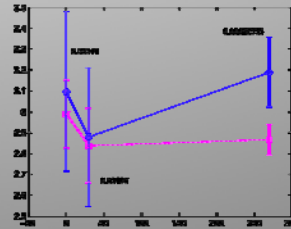
KIFAP3 kinesin-associated protein 3
(probe set 203333_at)



Gene POP5 (probe set 204839_at)



ABCC2 ATP-binding cassette
(probe set 206155_at)



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Team Effort

Jim Fuite, PhD Research Associate
Sadiq Alyanka PhD student

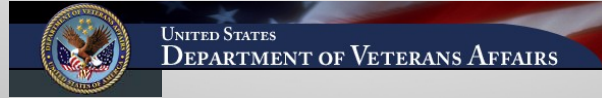
Andrea Kreitz Y3 Medical Student
Christina Yang Y2 Medical Student
Ann Aspler, MSc Y4 Medical Student
Carly Bolshin Y4 Medical Student

Helping training the next generation of
clinician



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Acknowledgement of Funding



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