ALTERED IMMMUNE FUNCTION IN GWI AND POTENTIAL THERAPIES

Nancy Klimas, MD, Mar Ann Fletcher, PhD and Gordon Broderick, PhD

UNIVERSITY OF ALBERTA

UNIVERSITY OF MIAMI

MIAMI VETERANS AFFAIRS HEALTHCARE SYSTEM

University of Miami/Miami VAMC CFS and GWI Research Team Leaders:

Nancy Klimas, MD Center Director, Clinical core
Mary Ann Fletcher, PhD Immunology/Biomarker
Gordon Broderick, PhD Computational Biology
University of Alberta
Barry Hurwitz, PhD Autonomic
Mike Antoni, PhD CBT/Stress response
John Gilmore,PhD Genomics

Funding: NIH ORWH /trans-NIH CFS working group; DOD OCMR; Veterans Affairs Research Administration; CFIDS Association of America; PANDORA; private donations

MODEL OF PATHOGENESIS

Genetic Predisposition

↓

Triggering event / infection

↓

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

↓

CFS/ME

Hormone	Examples of Immune Cells with Receptors
Gluccocorticoids	T and B-lymphocytes, neutrophils, monocytes/macrophages
Substance P	T and B-lymphocytes, eosinophils, mast cells, monocytes/macrophages
Neuropeptide Y	T-lymphocytes, monocytes/macrophages
Corticotropin Releasing Hormone	T and B-lymphocytes, mast cells, monocytes/macrophages
Prolactin	T and B-lymphocytes, granulocytes, precursor cells, monocytes/macrophages
Growth Hormone	T and B-lymphocytes, monocytes/macrophages
Catecholamines (epinephrine/norepinephrine)	T and B-lymphocytes, neutrophils, NK cells, monocytes/macrophages
Serotonin	T and B-lymphocytes, NK cells, monocytes/macrophages

Glaser and Kiecolt-Glaser, *Nature Reviews Immunology*, 2005

Mediators

- 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.

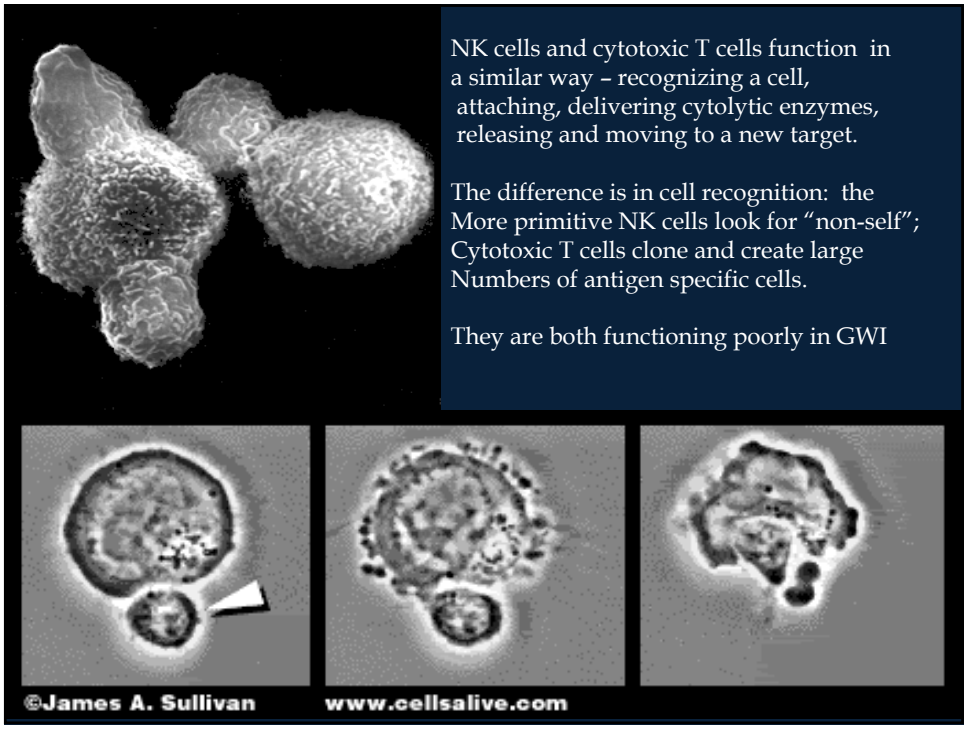
Immune abnormalities in GWI

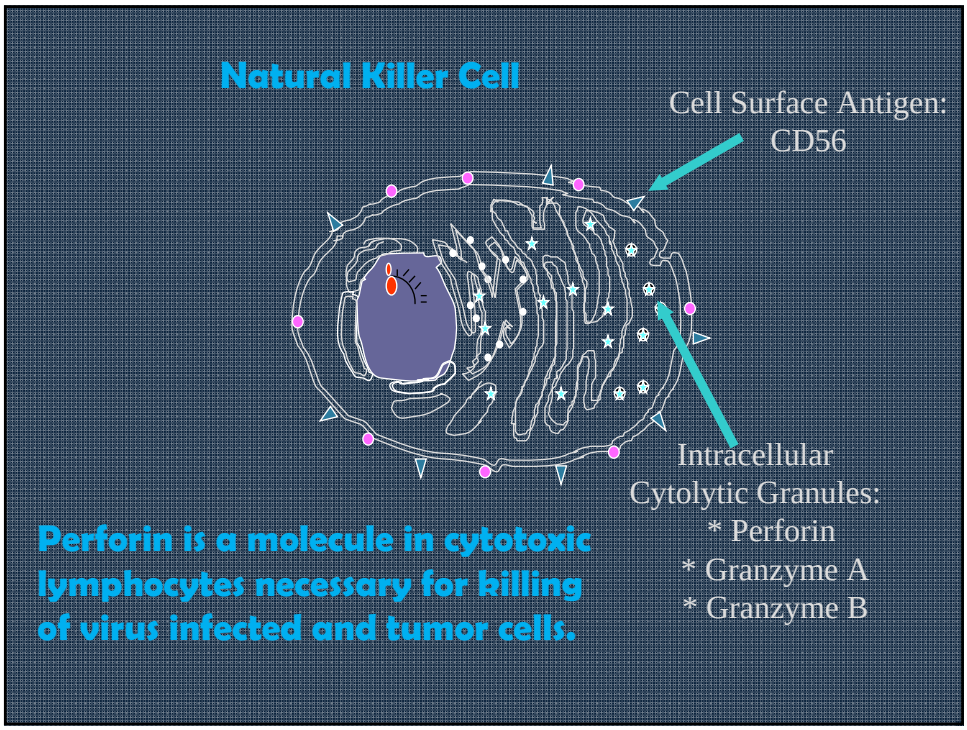
Immune Activation

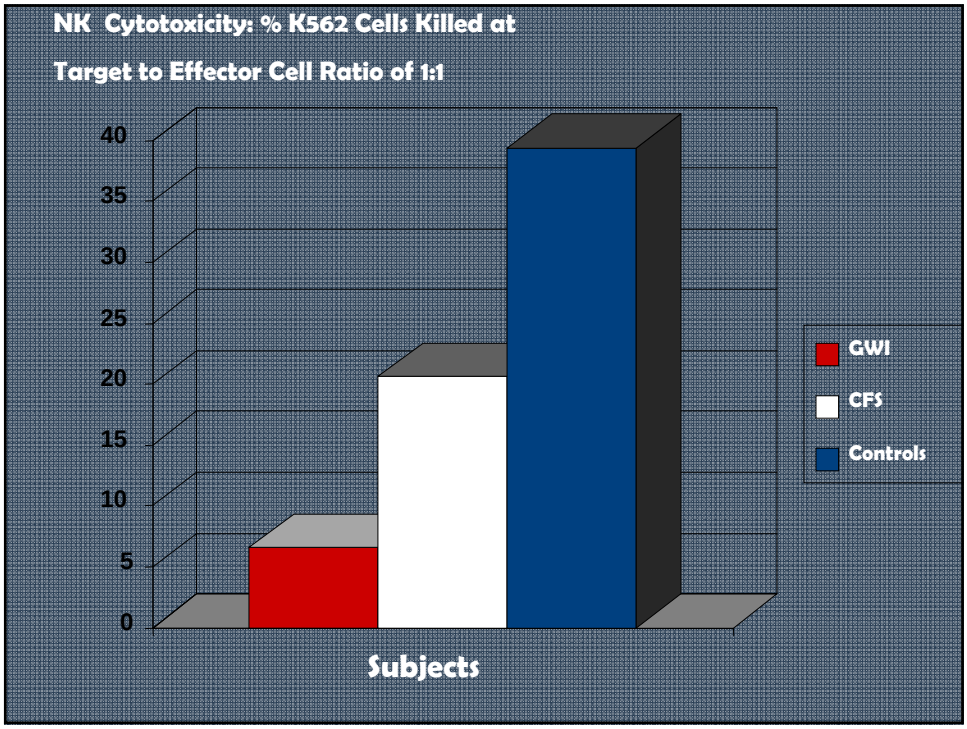
- DR, CD26, CD 38 expression
- TH2 cytokine shift
- Proinflammatory cytokines expression TNF-a, IL-1, IL6

Functional defects

NK Cell dysfunction
CD8 abnormalities
↓perforins, granzymes
Macrophage abnormalities
Antibody production

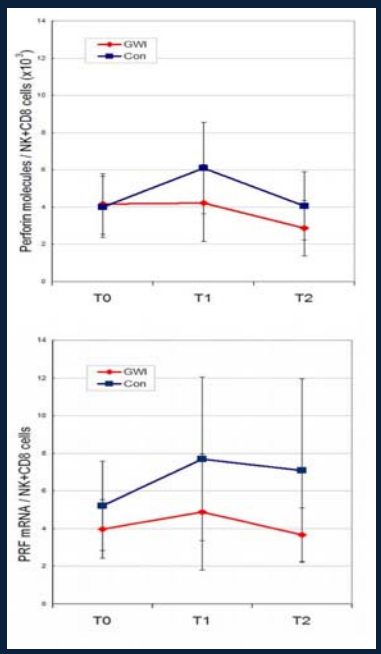

NK cells and cytotoxic T cells function in a similar way – recognizing a cell, attaching, delivering cytolytic enzymes, releasing and moving to a new target.
The difference is in cell recognition: the More primitive NK cells look for "non-self"; Cytotoxic T cells clone and create large Numbers of antigen specific cells.
They are both functioning poorly in GWI
©James A. Sullivan
www.cellsalive.com


Natural Killer Cell
Cell Surface Antigen: CD56
Intracellular Cytolytic Granules:
* Perforin
* Granzyme A
* Granzyme B
Perforin is a molecule in cytotoxic lymphocytes necessary for killing of virus infected and tumor cells.

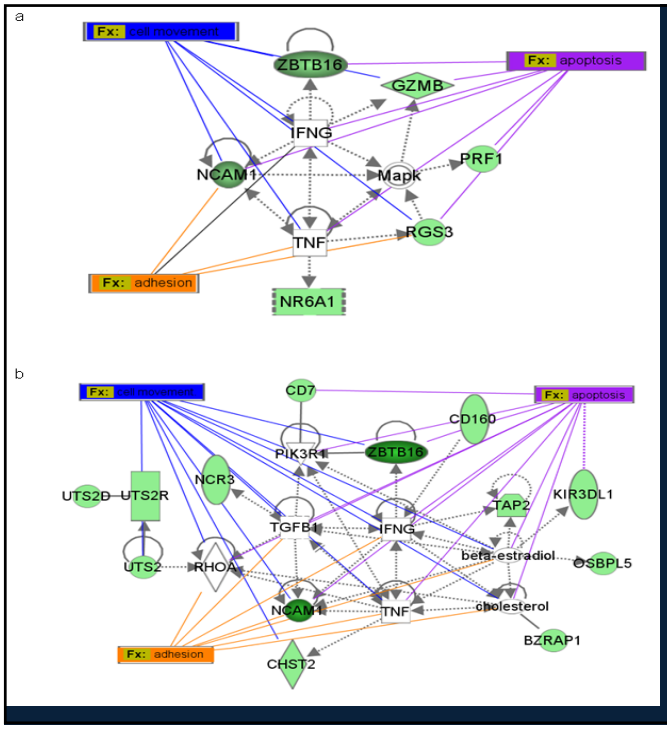


Change in perforin levels during an exercise challenge time series adjusted for the number of NK and CD8+ cells.

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).



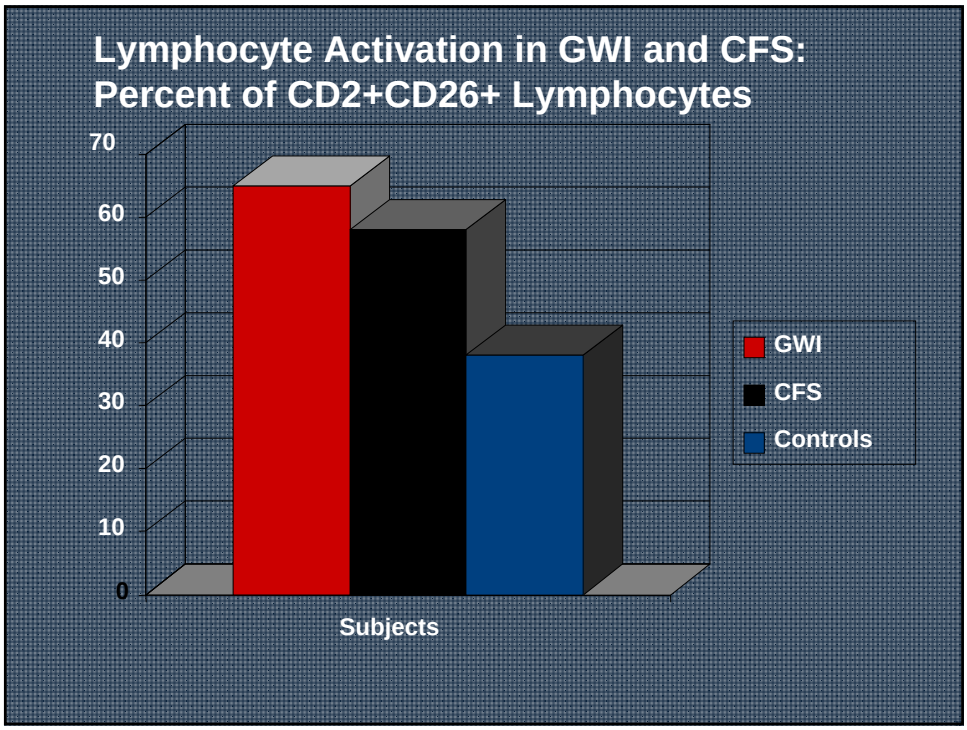
BMC Med Genomics. 2009; 2: 12. Published online 2009 March 5. Impaired immune function in Gulf War Illness Toni Whistler, Mary Ann Fletcher, William Lonergan, Xiao-R Zeng, Jin-Mann Lin, Arthur LaPerriere, Suzanne D Vernon, Nancy G Klimas



Genes regulating NK cell function map differentially in controls and GWI.

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

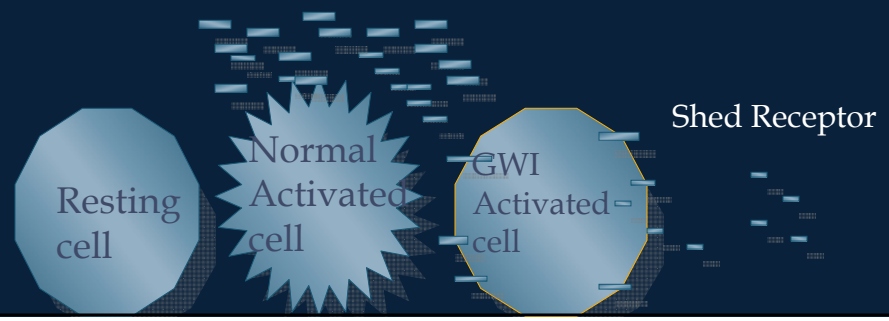
Genes regulating perforin and granzymes are also differentially expressed.



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.

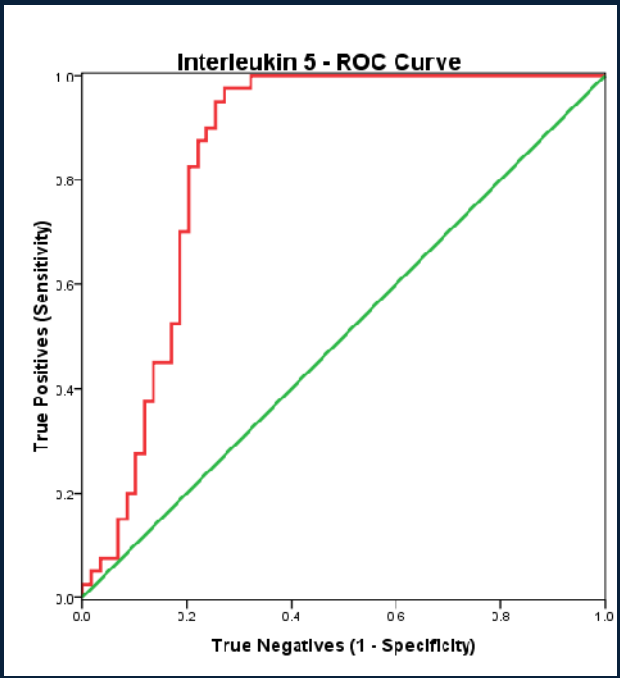


Neuropeptide-Y (NPY) helps to regulate of a large number of physiological and pathophysiological processes.

cardiorespiratory system
immune system
nervous system
endocrine system

Signal molecules Plasma levels	Ctrl	GWI	EGWI - Ectrl	P value
IL-6	12.60 (0.76)	3.45 (0.23)	9.15	0.00
IL-10	10.07 (0.85)	3.78 (0.19)	6.29	0.00
TNF-a	0.05 (0.00)	4.89 (0.19)	4.84	0.00
SCD26	2.13 (0.08)	6.94 (0.53)	4.81	0.00
NPY	7.71 (0.55)	12.23 (0.84)	4.52	0.00

PHA-stimulated blood culture	Ctrl	GWI	EGWI - Ectrl	P value
Saliva Cortisol	3.12 (0.15	6.10 (0.32)	2.98	0.00
IL-la	2.12 (0.12)	22.01 (1.68)	19.89	0.00
IL-5	3.39 (0.28)	13.06 (0.45)	9.67	0.00
IL-6	2.19 (0.18)	7.09 (0.46)	4.90	0.00
IL-10	11.75 (0.97)	6.88 (0.62)	4.87	0.00
TNF-a	2.00 (0.15)	10.23 (0.43)	8.23	0.00
IFN-g	10.16 (0.44)	10.33 (0.88)	0.16	0.76



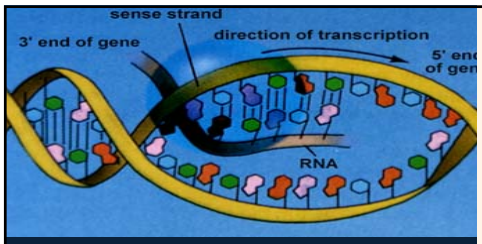
Conclusion

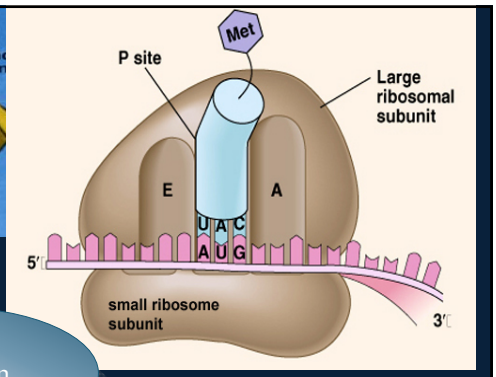
- The immune abnormalities seen in GWI include immune activation, poor cytotoxic cell function, cytokine regulatory disruptions and abnormalities of neuropeptide Y and cytokines that interface with autonomic, endocrine, and neurologic mediators.
- Many of the mediators seen are strong enough to be considered 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.

Gene Environment Interactions

Gene → Gene/ Environment → Environment

Downs Syndrome	Diabetes CFS GWI	Fracture bone

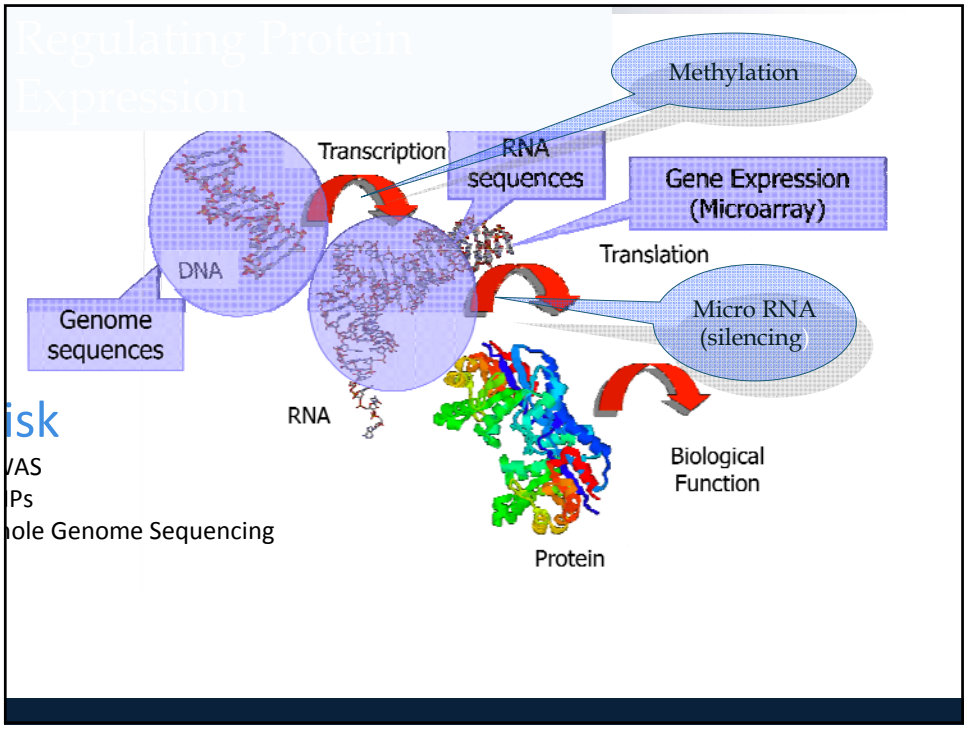




Silencers
Methylation,
miRNA

DNA → Transcription → Post transcription → Intracellular and extracellular Proteins

GWAS	Microarray gene expression	MicroRNA silencing


Regulating Protein Expression
Methylation
Transcription
RNA sequences
Gene Expression (Microarray)
Translation
DNA
Genome sequences
Micro RNA (silencing)
RNA
Biological Function
Protein
isk
VAS
Ps
ole Genome Sequencing