

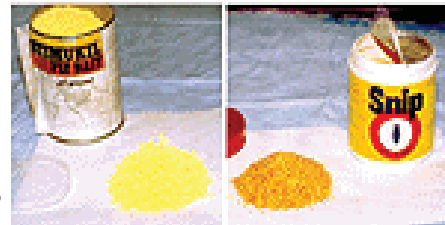
# How To Discussion: Environmental Exposure Assessment and Biomarker Development

Dr. Kimberly Sullivan  
Associate Scientific Director  
RAC GWVI

## How Were Pesticides Used in Gulf War Theatre?

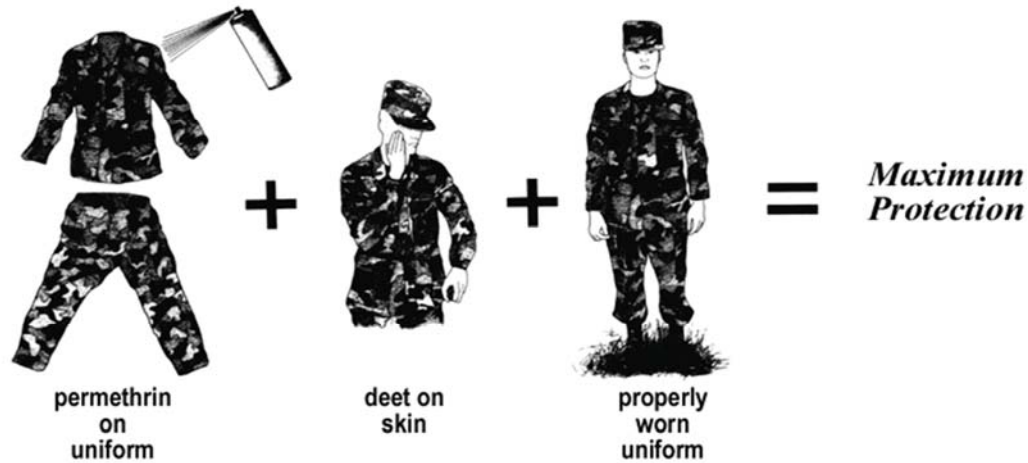
- Troops used pesticides for personal use on skin and uniforms and as:

- Insect repellants
- As area sprays and fogs
- In pest strips and fly baits
- As delousing agents for POWs



- Those who applied the pesticides were likely exposed to more pesticide products and at higher doses.
- Military installations used 1 million pounds of pesticides per year during the 1990s

## Field Guide for Maximum Protection



## How many pesticides were in Gulf War Theatre?

- Pesticides were used widely in the Gulf War to protect the troops from pests such as sand flies, mosquitoes and fleas that can carry infectious diseases.
- US forces used pesticides in areas where they worked, slept and ate. In fact, on any given day during their deployment GW veterans could have been exposed to at least 15 pesticide products of concern with 12 different active ingredients.
- A Health Risk Assessment conducted by DOD estimated that 43,000 GW veterans could have been overexposed to pesticides during the war.
- This was likely an underestimate since it didn't account for multiple exposures and potential additive and/or synergistic effects.

## ENVIRONMENTAL EXPOSURES - PESTICIDES OF POTENTIAL CONCERN

Repellents	Pyrethroids	Organophosphates	Carbamates	Organochlorines
DEET	Permethrin	Azamethiphos*	Methomyl	Lindane*
	D-Phenothrin	Chlorpyrifos*	Propoxur	
		Diazinon*	Bendiocarb*	
		Dichlorvos*		
		Malathion*		

Organophosphates are known to cause significant oxidative stress and can cause damage to mitochondria and are also known to damage axonal transport mechanisms by altering microtubules that carry organelles and cytoskeletal elements.

\*Current use phased out by EPA as part of the Food Quality Protection Act in 1996.

Source: DOD Environmental Exposure Report – pesticides

## OTHER ENVIRONMENTAL EXPOSURES

Exogenous vs. Endogenous Danger Signals





Other relevant  
GW Exposures?

Anti-nerve gas pills

Pyridostigmine Bromide  
(PB)



Khamisiyah Weapons Depot Detonations:  
Sarin/cyclosarin Exposure  
100,000 potentially exposed





## How Do We Measure GW Exposures?

- Self-report Surveys
- Wind pattern and exposure modeling
- Blood biomarkers for downstream effects?

# Self-reported Environmental Exposure Surveys

Survey	Outcomes
Kansas Environmental Exposure Survey	Assesses Gulf War exposures including PB, pesticides, DU, oil fires
Structured Neurotoxicant Exposure Checklist (SNAC)	Assesses Occupational exposures including military and non-military and hobby-related exposures

Available to researchers upon request

**GENERAL HEALTH INFORMATION:** (Please check the answer to these questions that best applies to you and your experiences)

## MILITARY SERVICE

Please answer the following questions regarding your service career. For each type of service you were in, please complete the questions across that row. If you did not belong to that service, check the "No" response in the left column and move on to the next row.

	Branch of service (check all that apply):	How many years total were you on duty?	What years were you in this service?	What was the highest rank you achieved?	What was your primary MOS and military job title?
1. Have you ever served on Active Military Duty? - Yes (if yes, continue with this row) - No (if no, skip this row and go to the next row)	- Army - Air Force - Navy - Marines - Other	<input type="text"/> <input type="text"/> (enter number of years on Active Duty in the box above)	List first and last year of service. From: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> To: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	- Enlisted - NCO - Officer	MOS: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> ↓ Military Job Title: _____ _____
2. Have you ever served in the Reserves? - Yes (if yes, continue with this row) - No (if no, skip this row and go to the next row)	- Army - Air Force - Navy - Marines - Other	<input type="text"/> <input type="text"/> (enter number of years on Reserve Duty in the box above)	List first and last year of service. From: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> To: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	- Enlisted - NCO - Officer	MOS: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> ↓ Military Job Title: _____ _____
3. Have you ever served in the National Guard? - Yes (if yes, continue with this row) - No (if no, go to question #4)	- Army - Air Force - Navy - Marines - Other	<input type="text"/> <input type="text"/> (enter number of years on National Guard Duty in the box above)	List first and last year of service. From: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> To: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	- Enlisted - NCO - Officer	MOS: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> ↓ Military Job Title: _____ _____

**OCCUPATIONAL EXPOSURE HISTORY:**

Please indicate if you have ever been exposed to any of the materials listed in the far left column. Include time spent in military service.

Type of exposure:	Examples of this type of exposure include:	Examples of work settings where exposure could occur:	Did you ever have a job where you were exposed?	If you think you were exposed or might have been, list the first and last years you were working that (or those) job(s)?	If yes, was this exposure during your military job, civilian job, or both?	Over that time, how often would you say you were exposed? <small>very often = every day                  often = 1-4 times/month                  sometimes = once/month to once 6 months                  rarely = once/year or less</small>	Did you wear personal protection when you were exposed?
<b>Solvents and Fuels</b>	Trichloroethylene; Benzene; Gasoline; Paint Thinners; Diesel Fuel; Formaldehydes	Auto body repair or painting; Auto mechanic	<input type="checkbox"/> Yes <input type="checkbox"/> Don't Know <input type="checkbox"/> No	From <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> To <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="checkbox"/> Military <input type="checkbox"/> Civilian <input type="checkbox"/> Both	<input type="checkbox"/> Very Often <input type="checkbox"/> Often <input type="checkbox"/> Sometimes <input type="checkbox"/> Rarely	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Sometimes
<b>Petroleum Combustion Products</b>	Gasoline, or Jet exhaust; Tint heaters; Oil/Chemical fires; Diesel Fuel	Auto/Truck mechanic; Oil delivery & services; Cold-weather camping	<input type="checkbox"/> Yes <input type="checkbox"/> Don't Know <input type="checkbox"/> No	From <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> To <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="checkbox"/> Military <input type="checkbox"/> Civilian <input type="checkbox"/> Both	<input type="checkbox"/> Very Often <input type="checkbox"/> Often <input type="checkbox"/> Sometimes <input type="checkbox"/> Rarely	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Sometimes
<b>Lead</b>	Lead in paint; Soldering; Leadshot	Painting; Bridge repair; Radiator repair; Firing range	<input type="checkbox"/> Yes <input type="checkbox"/> Don't Know <input type="checkbox"/> No	From <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> To <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="checkbox"/> Military <input type="checkbox"/> Civilian <input type="checkbox"/> Both	<input type="checkbox"/> Very Often <input type="checkbox"/> Often <input type="checkbox"/> Sometimes <input type="checkbox"/> Rarely	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Sometimes
<b>Other Metals</b>	Mercury; Arsenic; Cadmium	Thermometer makers; Copper smelting; Mining; Pesticide workers	<input type="checkbox"/> Yes <input type="checkbox"/> Don't Know <input type="checkbox"/> No	From <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> To <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="checkbox"/> Military <input type="checkbox"/> Civilian <input type="checkbox"/> Both	<input type="checkbox"/> Very Often <input type="checkbox"/> Often <input type="checkbox"/> Sometimes <input type="checkbox"/> Rarely	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Sometimes
<b>Pesticides</b>	DDT; Diazinon; Malathion; Chlordane	Commercial farming; Pesticide application	<input type="checkbox"/> Yes <input type="checkbox"/> Don't Know <input type="checkbox"/> No	From <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> To <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="checkbox"/> Military <input type="checkbox"/> Civilian <input type="checkbox"/> Both	<input type="checkbox"/> Very Often <input type="checkbox"/> Often <input type="checkbox"/> Sometimes <input type="checkbox"/> Rarely	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Sometimes

**HOBBIES AND NON-OCCUPATIONAL EXPOSURES:** (Please indicate if you have ever performed any of these activities in which you might have been exposed)

	Have you participated in this activity frequently over any 6 month period?	If yes, did you wear any personal protection during these activities such as masks, boots, gloves, or other protective equipment?
1. Painting or Renovating your home	<input type="checkbox"/> Yes, within the past 10 years <input type="checkbox"/> Yes, but more than 10 years ago <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Sometimes
2. Furniture Refinishing	<input type="checkbox"/> Yes, within the past 10 years <input type="checkbox"/> Yes, but more than 10 years ago <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Sometimes
3. Auto Body Work	<input type="checkbox"/> Yes, within the past 10 years <input type="checkbox"/> Yes, but more than 10 years ago <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Sometimes
4. Work with Glues, Solvents, or Chemicals (such as those used in model building, fiberglass repair, etc.)	<input type="checkbox"/> Yes, within the past 10 years <input type="checkbox"/> Yes, but more than 10 years ago <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Sometimes
5. Pesticides while Gardening or Farming	<input type="checkbox"/> Yes, within the past 10 years <input type="checkbox"/> Yes, but more than 10 years ago <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Sometimes
6. Jewelry Making, Pottery work, Studio painting	<input type="checkbox"/> Yes, within the past 10 years <input type="checkbox"/> Yes, but more than 10 years ago <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Sometimes



### Kansas Gulf War Veterans Health Project

5. In August, 1990, what was your branch of service?      Army      Navy      Air Force      Marines
6. In August, 1990, were you in the enlisted ranks, or an officer?      Enlisted      Officer
7. In August, 1990, were you in the Regular military, the Reserves, or the National Guard?      Regular      Reserves      National Guard
8. In general, how would you describe your health *before* you went to the Persian Gulf?      Excellent      Good      Fair      Poor
9. In general, how would you describe your health now?      Excellent      Good      Fair      Poor
10. Were you a regular smoker before you deployed to the Persian Gulf region?      NO      YES
11. Were you a regular smoker while you were in the Persian Gulf region?      NO      YES
12. Are you currently a regular smoker?      NO      YES

### Questions About Your Military Service Between August, 1990 and July, 1991

1. Did you deploy to the Persian Gulf region any time between August, 1990 and July, 1991?      NO      YES
2. In what month and year did you first arrive in the region?      \_\_\_\_\_ (mo)      \_\_\_\_\_ (yr)
3. In what month and year did you last leave the region?      \_\_\_\_\_ (mo)      \_\_\_\_\_ (yr)
4. While you were in the Persian Gulf region, did you experience any of the following?

*[Please mark NO or YES for each]*

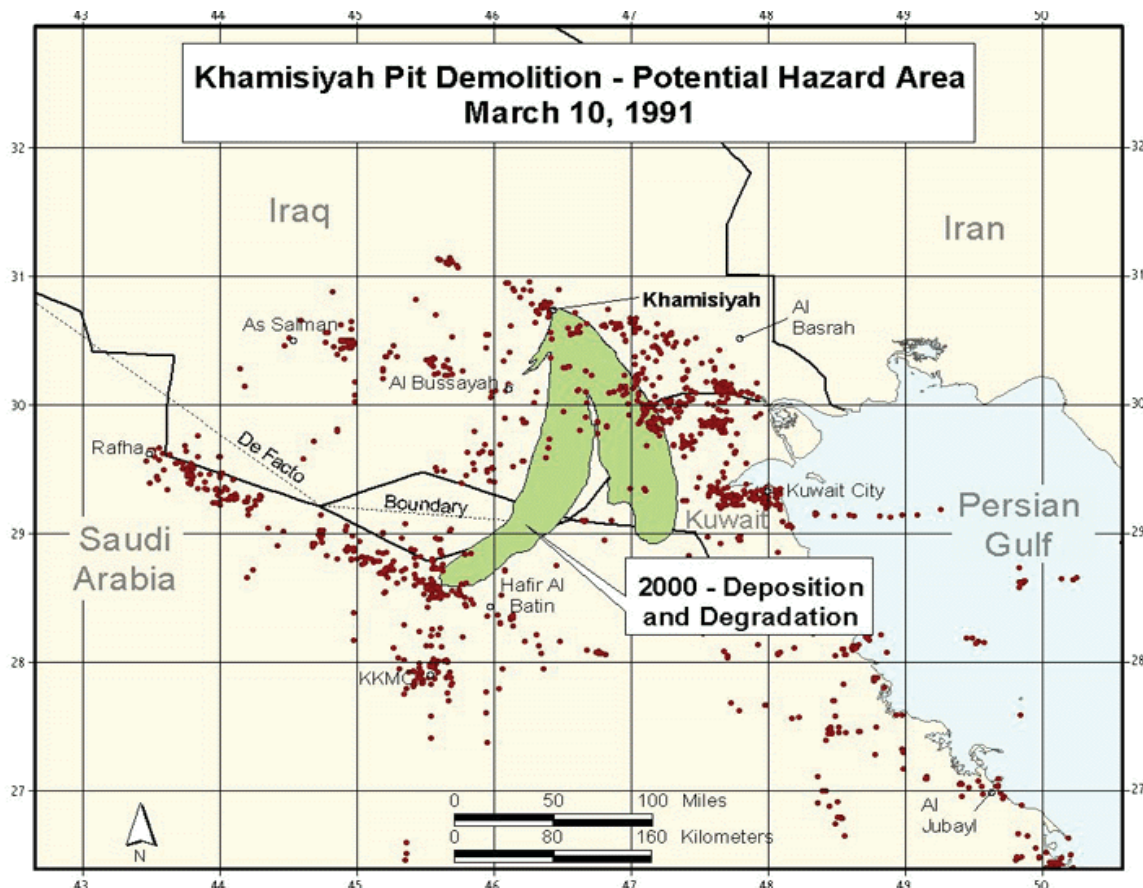
	NO	YES	IF YES, About how many days?		
			1 - 6 days	7 - 30 days	31 days or more
Saw smoke from oil well fires	<input type="radio"/>	<input type="radio"/> →	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Heard chemical alarms sounded	<input type="radio"/>	<input type="radio"/> →	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Had SCUD missile explode within one mile of you	<input type="radio"/>	<input type="radio"/> →	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Was directly involved in ground combat	<input type="radio"/>	<input type="radio"/> →	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Was directly involved in air combat	<input type="radio"/>	<input type="radio"/> →	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Saw American or Allied troops who had been badly wounded or killed	<input type="radio"/>	<input type="radio"/> →	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Saw Iraqis or civilians who had been badly wounded or killed	<input type="radio"/>	<input type="radio"/> →	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Came into contact with prisoners of war	<input type="radio"/>	<input type="radio"/> →	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Saw dead animals	<input type="radio"/>	<input type="radio"/> →	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>



# Dose Exposure Models

Outcomes	Results Summary
Sarin/cyclosarin dose-exposure modeling	Modeled wind patterns and plume estimates at Khamisiyah in March 1991 listed by DOD Force Health Protection and Readiness Office
Oil well fire dose modeling	Modeled wind patterns and dispersion estimates near oil well fires during war by USACHPPM. Gives individual low or high exposure estimates.

<https://usaphcapps.amedd.army.mil/gwf/entry.asp>  
[http://www.gulfink.osd.mil/library/kham\\_info.jsp](http://www.gulfink.osd.mil/library/kham_info.jsp)



# Data Analysis of Exposure Estimates

## RAC Report Recommendations for Exposure Data Analysis

**Improved Methodology Guidelines for Epidemiological Research:** Should include systematic methods for assessing symptoms and other health outcomes, evaluation of health outcomes in subgroups of importance (exposure, location), use of analytic methods that control for multiple exposures (confounding effects and synergistic effects)

This could include covariate analyses, interaction effect analyses and structured equation modeling (SEM) analyses. SEM is a technique for testing and estimating causal relationships and hypotheses using a combination of statistical data and qualitative assumptions.

Future meeting speakers will be address these potential analytic approaches

# Biomarker Development

## Biomarkers

Biomarkers are quantitative biological measures that can facilitate the diagnosis of Gulf War Illness and allow monitoring of its progress and a patients' response to treatment.

Biomarkers of GWI may represent molecular or cellular events that can be identified as a link to a specific environmental exposure or to a health outcome.

Results from imaging technologies can also be considered surrogate biomarkers when they associate with disease or disease progression.

# Biomarker in GWI

## Objective biological measures

There are currently no laboratory tests that accurately diagnose individual patients with GWI however research studies have identified objective biological measures that distinguish between groups of ill GW veterans and controls.

- These areas include measures of brain structure and functioning , autonomic nervous system functioning, neuroendocrine function and immune functioning.
- These biological findings are generally considered preliminary because they have been found in a limited number of studies using different methods and measures.
- These findings have been useful because they provide important clues and insights into the underlying pathobiology of GWI and provide targets for development of useful biomarkers
- These significant findings across multiple body systems need to be validated in multiple studies with similar methods and within the same individuals.

## Path to Biomarker Development

### As FDA Guidelines Suggest:

The path to development of biomarkers has also been summarized by FDA as including:

**Biomarker Discovery**

**Biomarker Qualification**

**Biomarker Application**

# Biomarker Discovery

## As FDA Guidelines Suggest:

**Discovery** of a differentiating signature in a measurement as a candidate biomarker.

In-depth investigations of the mechanisms of action and biological pathways the candidate biomarker reflects. This is the best source of information on the likely relevance, specificity and robustness of the candidate biomarker.

# Biomarker Qualification

## As FDA Guidelines Suggest:

Development of a robust and practical method for biomarker detection

Proof-of-principle in controlled experimental settings

Establishing that the biomarker adequately selects and characterizes the presence and / or severity of the outcome of interest in specific patient populations

Understanding the candidate biomarkers' clinical performance with regard to the level of sensitivity and specificity achieved under a specific context of use.

Identification of clinical factors which might interfere with biomarker interpretation

# Biomarker Application

## As FDA Guidelines Suggest:

Use of the biomarker to predict disease progression / success of therapeutic interventions etc. in the context for which it was qualified.

## Biomarkers of Exposure



# Exposures and Health Outcomes: Neurological/Neuropsychological

Study	Exposure	Method	Health Outcomes
Chao, 2010	Sarin and cyclosarin	MRI, neuropsychological testing	Sig. reduced gray matter and hippocampal volumes
Chao, 2011	Sarin and cyclosarin	MRI, neuropsychological testing	Sig. reduced total gray and white matter volume
Toomey, 2009	PB, pesticides, vaccines, IG injections, oil well fire smoke	Neuropsychological testing	Deployed GWV had sign. lower scores on tests of verbal memory, verbal learning, motor speed, and attention

Significant findings in 3/3 studies

## RAC-GWVI Biomarkers of Exposure

**Studies that characterize effects of neurotoxic exposures associated with Gulf War illness.** Due to the consistency of findings relating Gulf War illness to neurotoxic exposures during the war, the Committee gives high priority to studies that further characterize specific effects of Gulf War related to neurotoxic exposures, and recommends the following research:

- Studies that utilize animal models to characterize persistent molecular, cellular, systemic, and behavioral effects of individual and combined exposure to pyridostigmine bromide, pesticides and insect repellants used in the Gulf War, and low-level sarin.
- Studies that utilize animal models to characterize persistent effects of GW-related exposures, alone and in combination, on central proinflammatory processes and their biological mediators in the central nervous system and target organs.

# IOM Recommendations

## IOM biomarker statement

“Many of these symptoms (Gulf War) are difficult to categorize as they have no known cause, no objective findings on clinical examination, no diagnostic biomarkers, no known tissue pathology, and no curative therapy. The inadequate basic understanding of the root cause of these symptoms highlights the limitations of current medical science and clinical practice. The (IOM) committee recognizes that symptoms that cannot be easily quantified are sometimes dismissed—incorrectly—as insignificant, and that they receive inadequate attention—and funding—by the medical and scientific establishment.”

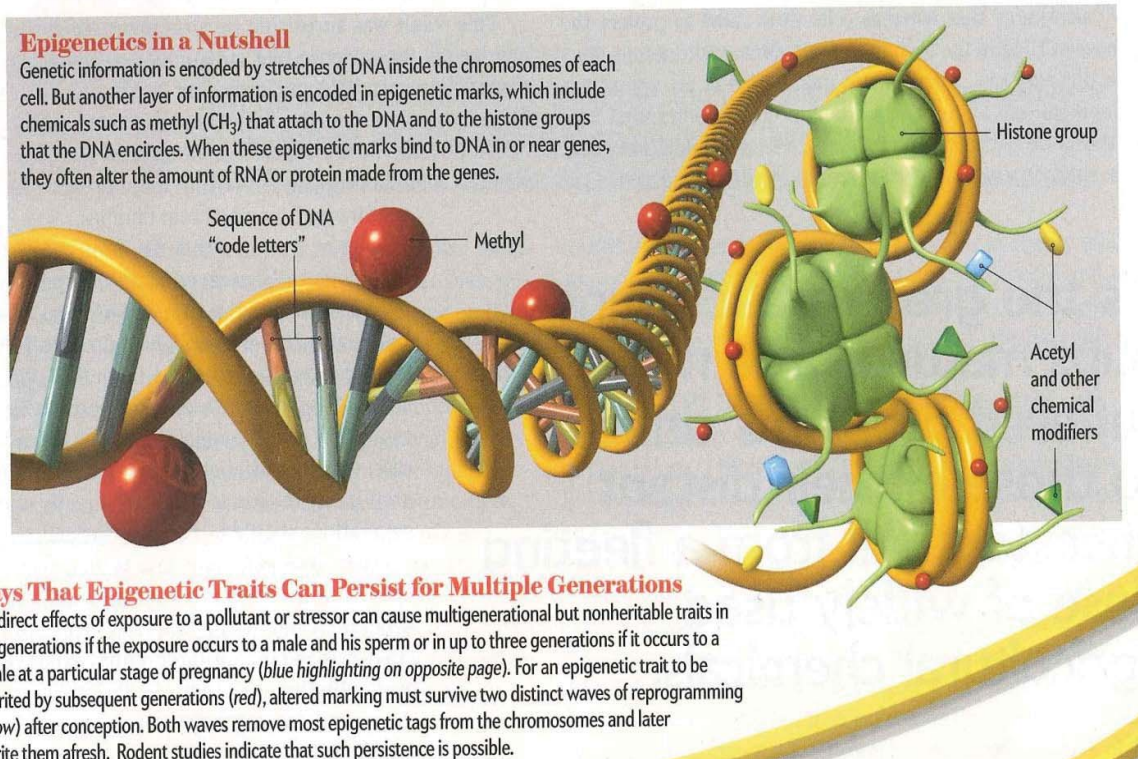
The committee recommends rigorous, adequately powered studies to identify biomarkers that distinguish Gulf War veterans who have persistent multisymptom illness (MSI) from healthy deployed or nondeployed veterans.

# IOM Recommendations

## Suggested Biomarker Research Areas:

- Inherited genetic variants
- Molecular profiles of gene expression
- Other epigenetic markers (e.g., modified DNA structures)
- Specific viral exposures
- Signature of immune activation
- Brain changes detected through imaging

# Epigenetics and DNA Methylation



## RAC GWVI Recommendations

### Recommendations

Clear, operational case definitions are important for this work

Theater exposures, age and other variables likely moderate pathobiological effects and should be carefully addressed in research

Gender differences may play a role in pathobiological expression of GWI and its effects. Gender should be considered whenever possible in mechanistic/treatment research of GWI

Exploratory probes in genetics, metabolomics, lipidomics and proteomics may yield useful information that can lead to more focused research

Epigenetic and genetic approaches to research on GWI pathobiology likely to be informative



## RAC GWVI Recommendations2

### Recommendations

Standard protocols for sample collections should be established and followed in research that uses biological specimens in order to expedite exploratory and hypothesis-driven research.

Increased emphasis should be placed on the study of alterations in regulatory dynamics both within and across the principal regulatory axes, including the endocrine, immune and nervous systems. These should include response to standardized challenges at different time scales, i.e., acute response to exercise, circadian rhythm, and monthly cycles as well as long-term illness progression.

Animal models may be appropriate to investigate mechanistic hypotheses and illness or exposure effects.

## Biologic Biomarkers in GWI

Nancy Klimas, MD

Director, GWI and CFS/ME Program

Miami VAMC

## Biomarkers purpose

- Identify the group
- Identify a subgroup
- Predict severity
  
- May also provide a target of intervention
- Shared biomarkers in animal models increase confidence in human translation

## GWVI biomarkers

- Neuroimaging
- Clinical objective measures
- Laboratory based markers

# Neuroimaging

MRI for anatomic differences

White matter decreases (several studies)

White and grey matter decrease

Localized areas with abnormal findings

fMRI assessing brain function using cognitive tasks

fMRI/MRS assessment of localized CNS lactate

Am J Transl Res. 2013; 5(2): 212–223. **Prefrontal lactate predicts exercise-induced cognitive dysfunction in Gulf War Illness**

PET scans assessing for metabolic function, newer scans capable of looking at neuro-inflammation

# Clinical Objective Markers

- PE findings (such as tender point evaluation)
- Objective markers of cognitive function (attention, concentration, reaction time)

# Laboratory Studies of Biomarkers in GWI

Blood studies the most common

Attention to compartment: blood, spinal fluid, GI

Many different studies looking at immune, autonomic, neuroendocrine, toxic substances as well as mitochondrial studies, detoxification pathways, and most recently an effort at large proteomic, genomic and sequencing methods with computational “big data” models to deal with the complexity

Increasing the signal with a challenge

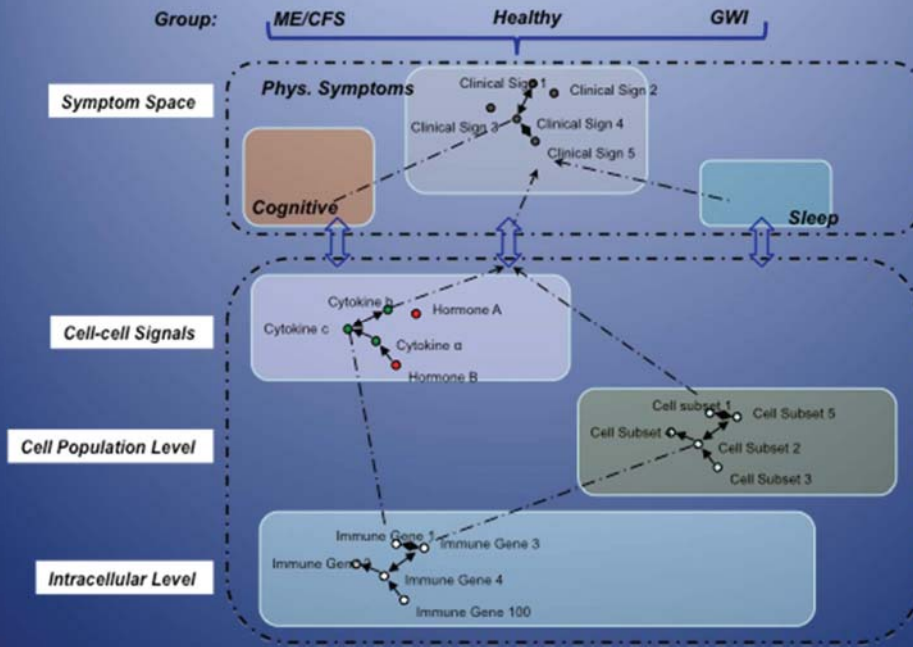
## Identifying Biomarkers with Therapeutic Implications

- ME/CFS and GWI: both complex disorders with overlapping constellations of symptoms
- Affecting several major regulatory systems
- Using an exercise stressor model (rest, peak VO<sub>2</sub>, 4-hrs post effort)
- Comprehensive survey of endocrine-immune function: gene, cell and immune protein levels
- Systems biology approach, to map gene expression to pathways to symptoms in each diagnostic group

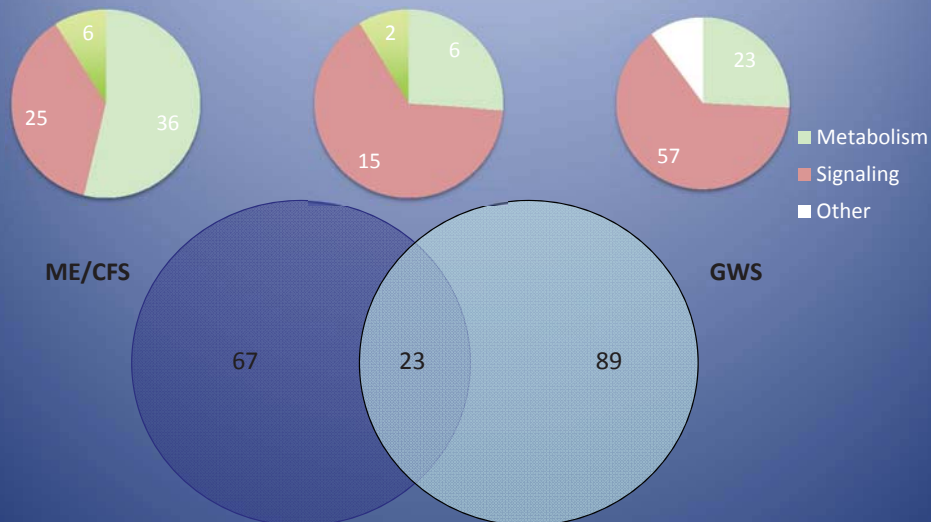


*Mapping mechanisms of illness to identify most effective intervention point(s)*

### Where Should We Look?

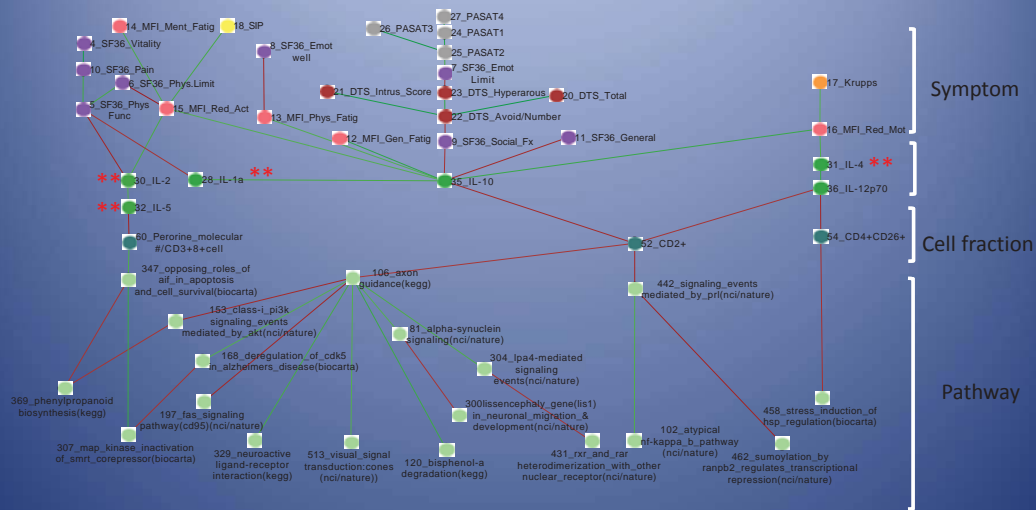


### Differences in Pathway Activity in ME/CFS



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

## Brain Immunity and Symptom Severity



- Consistent with observed correlation of IL-2, 4, 5 with GWI severity
- Also find previously identified IL-1a...

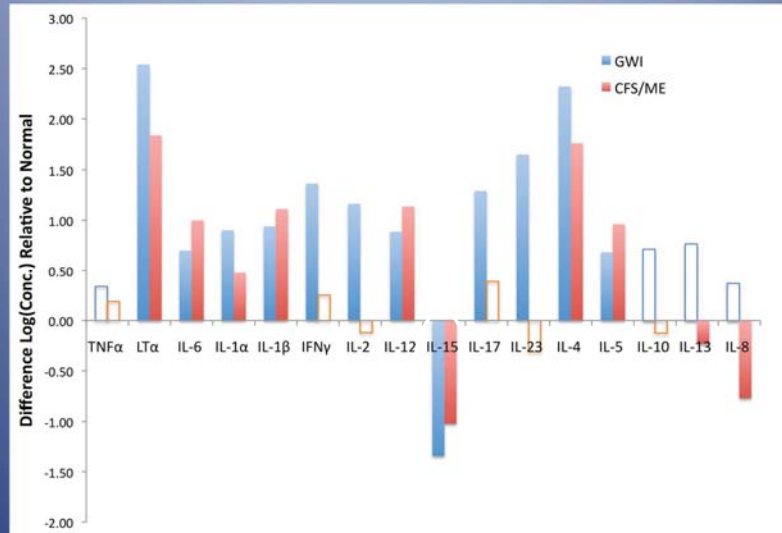
<sup>1</sup> Broderick et al., Altered immune pathway activity under exercise challenge in Gulf War Illness: An exploratory analysis. Brain Behav Immun. 2013 Feb;28:159-69.

### Effects of Exercise on Biomarkers\*

Biomarker	Friedman Test Result: (significant values of $p$ in bold)	
	GWI	HC
%CD26+CD2+ T &NK cells	<.000 (↓)	<.000 (↓)
rMolCD26/CD2+/ T & NK cell	<b>0.015</b> (↑)	<.000 (↑)
NPY (pMol/L plasma)	<b>0.049</b> (↑)	<.000 (↑)
IL-1 $\alpha$ (pg/ml plasma)	0.063 (↑)	0.600
IL-5 (pg/ml plasma)	<b>0.038</b> (↑)	0.218
IL-6 (pg/ml plasma)	0.675	<b>0.008</b> (↑)
IL-10 (pg/ml plasma)	<b>0.033</b> (↑)	<b>0.001</b> (↑)
IL-12p70 (pg/ml plasma)	0.219	<b>0.002</b> (↑)
TNF $\alpha$ (pg/ml plasma)	0.150	<b>0.007</b> (↑)
rMolPerforin/NK cell	<.000 (↑)	<.000 (↑)
NKCC (%)	<b>0.040</b> (↑)	<b>0.001</b> (↑)

\*35 GWI veterans with 35 carefully matched gulf war era controls before during and 4 hours after a short exercise challenge to VO2max (an average of 8 minutes).

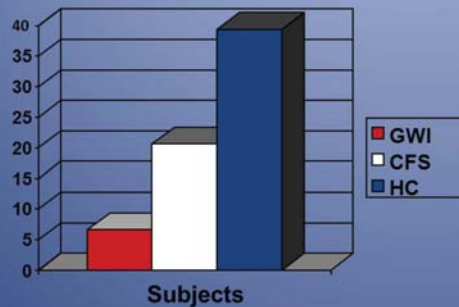
# Preliminary Data: Cytokines



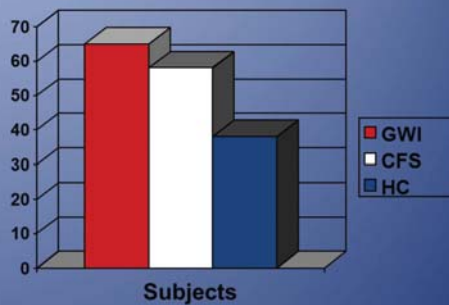
- Significant changes across a broad range of markers
- IFN $\gamma$ , IL-2, IL-17, IL-23 specific to GWI; IL-8, IL-13 unique to CFS

# Preliminary Data: Immune Activity

NKCC (whole blood bioassay\*)



CD26+CD2+ Lymphocyte activation (cytometry)\*

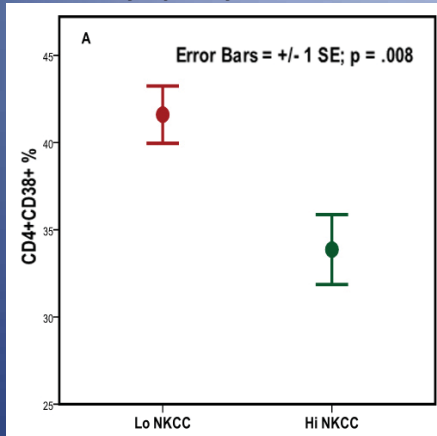


- Low NKCC Associated with High T Cell Activation & Low T Cell Function
- Chronic Immune Cell Activation & Dysfunction

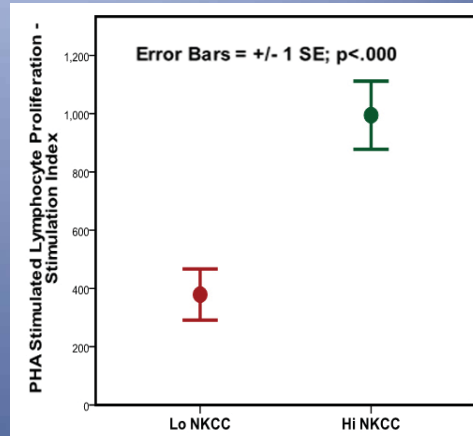
\*Klimas, et al, 1990; Fletcher, et al, 2010; Whistler, et al, 2009; Maher, et al, 2005; Fletcher, et al, 2002

## Low NKCC Associated with High T Cell Activation and Low Function

### T Lymphocyte Activation



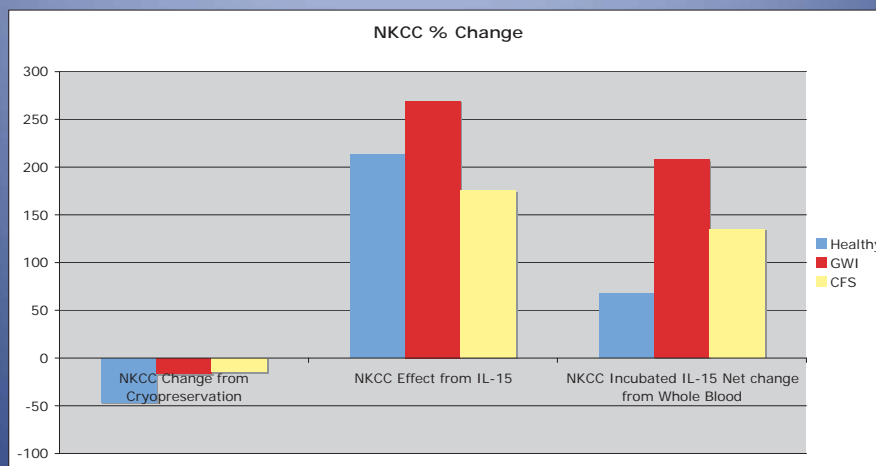
### T Lymphocyte Function



### NKCC vs % HLA/DR+T Cells

Correlation	-.417
Sig. (2-tailed)	<.0001
N	72

## From Biomarker to therapeutic target: the cytotoxic cell



- NK cell populations are stable across conditions
- GWI more *responsive*: increase in NK cell fitness, not expansion.

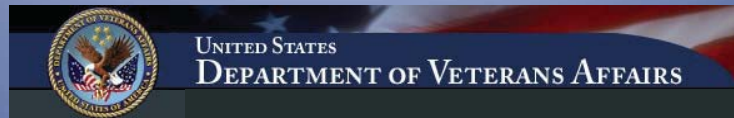


## Hot off the Press

NK cell function in GWI is much more responsive to IL-15 than in CFS. However in both cases IL-15 treatment will allow GWI and CFS to match or exceed NK cell functioning untreated controls.

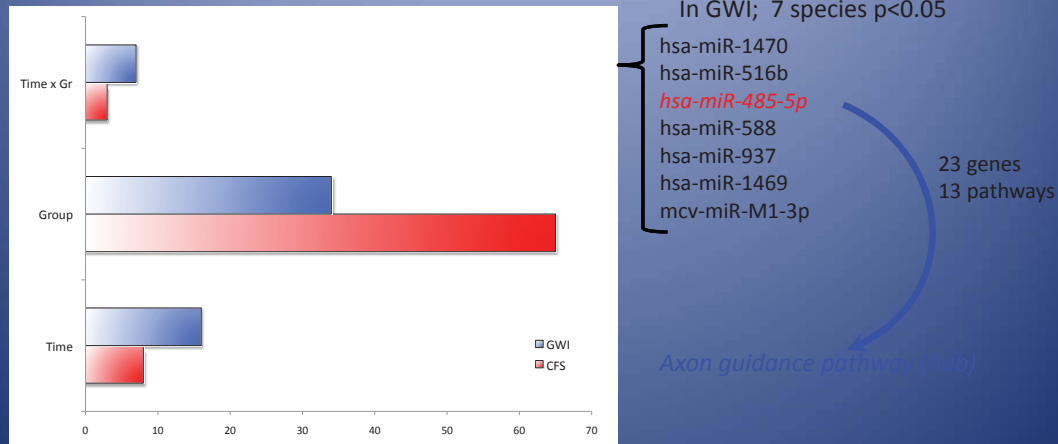
In terms of cytokine signaling patterns, we see similar responses. Basically treatment with IL-15 reduced the topological distance between GWI and normal healthy (untreated) by over 30%. The same IL-15 treatment reduced the distance between CFS cytokine signaling patterns and normal patterns by 15-20%. These are very preliminary numbers.

## Acknowledgement of Funding



### Study 1: Therapeutic Roadmap of ME/CFS: dynamics of mRNA and miRNA profiles in relapse lead to treatment (Klimas, P.I.)

Rationale AIM 1, 2: Ongoing work shows broad changes in metabolic pathway activation and miRNA is a key transcriptional regulator of these pathways

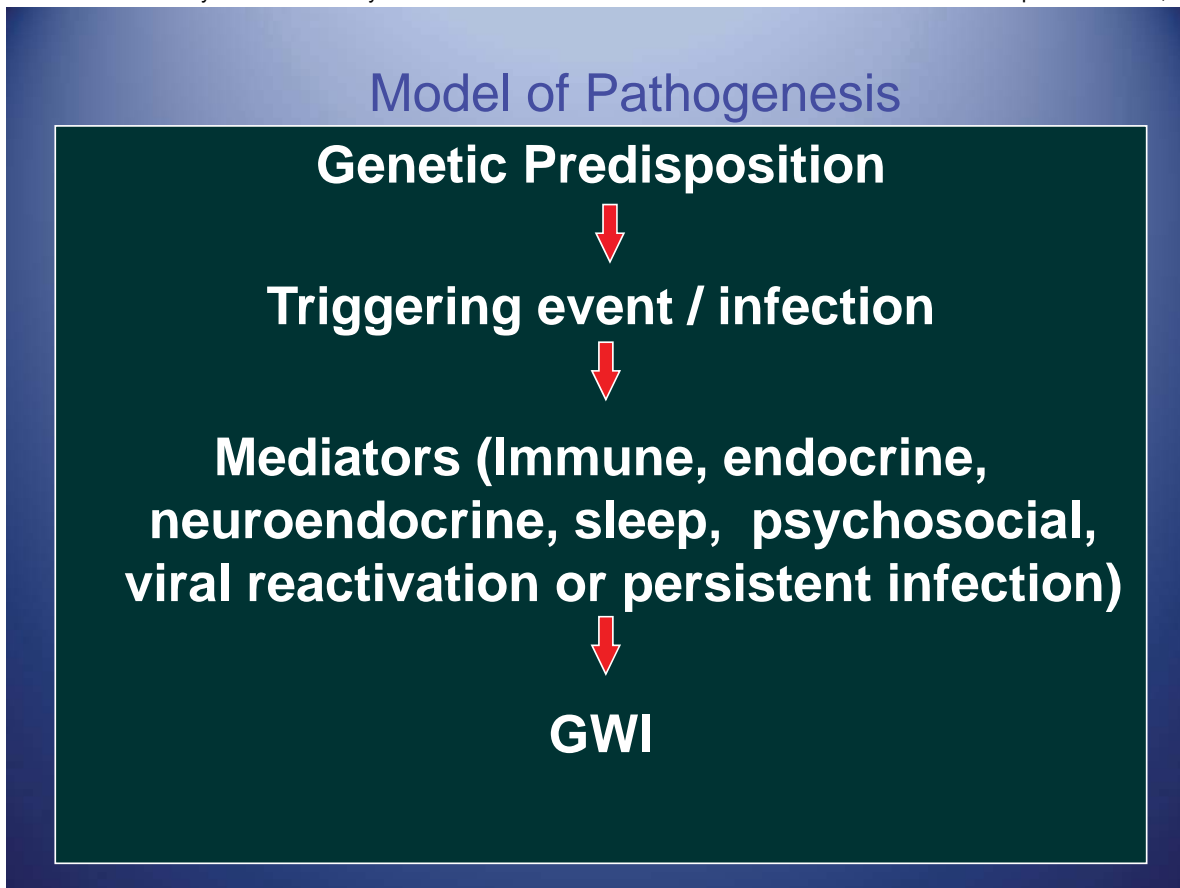


Preliminary work: Surveyed > 700 miRNA at 3 points during exercise; significant time-group effects; *confirmed suppression of hub pathway in GWI*

## The role of infectious disease in GWI

Not a recent focus of research, but new tools bring infectious diseases back to the table

In a system that has demonstrable poor cytotoxic function, consideration of co-morbid infection should be considered



## In what ways might an infection play a role in GWI

- Triggering infections inducing downstream events
- Persistent infections
- Recurrent infections
- Reactivation of latent infections
- Microbiome shifts and molecular mimicry inducing autoimmunity

## In what ways might an infection play a role in GWI

- **Triggering infections inducing downstream events**
- Persistent infections
- Recurrent infections
- Reactivation of latent infections
- Microbiome shifts and molecular mimicry inducing autoimmunity

## Infections endemic to the middle east

- These included Q fever, hepatitis, malaria, brucellosis, West Nile virus, malaria, shigellosis, and leishmaniasis, among others.
- Laboratory tests from all branches indicated that the major causes of diarrheal diseases in theater were enterotoxigenic E. coli and shigella sonnei.

## Triggering/acute infections

- Risk in Theater:
- Sand Flea vector – leishmaniasis
- Water borne illness – gastroenteritis: e coli, shigella reported 2-3 fold higher than non deployed
- Airborne illness – tight crowded quarters, viral syndromes reported at high levels airborne fungal illness, desert conditions

Germ warfare – anthrax; others – real time surveillance found no exposures

## Gastroenteritis and Irritable Bowel Syndrome

- Tuteja et al. (2008) studied 247 Gulf War deployed and nondeployed veterans from Salt Lake City deployed between 1990–1991
- Compared IBS rates before deployment (5.8%) to during deployment (38.9%;  $p = 0.03$ ) and this continued after deployment 18 years later (33.6%).
- A history of an enteric infection was a risk factor for developing IBS (OR 3.6, 95% CI 1.9-6.9) now called postinfectious IBS.

- The incidence of acquiring an acute gastroenteritis among deployed veterans is higher than nondeployed veterans, over 50% in some series (IOM, 2007).
- Deployed veterans who are exposed to an infectious gastroenteritis are at greater risk to be later diagnosed with IBS (Pulling et al., 2008; Riddle et al., 2009; Tuteja et al., 2008).
- Microscopic inflammation in IBS is associated with increased cytokine activity and mast cell degranulation that produces visceral hypersensitivity and abdominal pain (Barbara et al., 2004; Chadwick et al., 2002).
- Postinfectious IBS symptoms are facilitated by psychological distress via central nervous system (such as the hypothalamic-pituitary adrenal axis) effects on mucosal inflammation and enhanced pain via anterior cingulate cortex activation (Barbara et al., 2008; Drossman, 1999; Dunlop et al., 2003; Gwee et al., 1999).

## In what ways might an infection play a role in GWI

- Triggering infections inducing downstream events
- **Persistent infections**
- Recurrent infections
- Reactivation of latent infections
- Microbiome shifts and molecular mimicry inducing autoimmunity

## Persistent Infections

- Bacteria - **Mycoplasma, chlamydia, Q fever (brucella), rickettsia**, mycobacter
- Amoeba (typically causes liver abscess, diagnosable)
- Parasites (typically GI, diagnosable)
- Malaria (would have a classic presentation)
- Fungal diseases (would present in chest), **women report higher vaginal yeast infections than nondeployed**
- Viral acute infections in theater that could persist (hepatitis, live vaccine contaminants or attenuated species)
- Novel infections, unidentified for lack of methodology

## **Mycoplasma, chlamydia, Q fever (brucella), rickettsia**

- Mycoplasma extensively studied, large VA CSP placebo control study concluded the presence of mycoplasma by PCR did not predict disease or severity, eradication did not predict recovery.
- Chlamydia less well studied, studies have been hampered by lab contamination and false results, however, as it responds to the same AB as mycoplasma, the mycoplasma study that showed no improvement with AB discourages further study.

## Q fever, Rickettsia

- Q fever thought to be readily diagnosable, but newer data suggests serology data may be flawed
- Rickettsia sp also difficult to definitively diagnose
- Newer methodologies that identify pathogens by sequence data or multiple probes could improve our understanding of persistent infection

## In what ways might an infection play a role in GWI

- Triggering infections inducing downstream events
- Persistent infections
- **Recurrent infections**
- Reactivation of latent infections
- Microbiome shifts and molecular mimicry inducing autoimmunity



## Repeated infection: UTI

- In the first wave of the National Survey of Gulf War Veterans and Their Families conducted in 1995 sampled 15,000 Gulf War deployed veterans and 15,000 nondeployed era veterans (Kang et al., 2000)(75% response)
- Gulf War veterans reported bladder infections (difference in prevalence proportions 1.54%, 95% CI 1.49-1.59)
- A follow-up survey was conducted in this same population in 2005 (Kang et al., 2009). The prevalence of self-reported conditions 14 years after the war was re-examined among 6111 Gulf War veterans (40% response) and 3859 Gulf War era veterans (27% response). An increased prevalence of bladder infections (prevalence ratio 1.32, 95% CI 1.17-1.49) was observed among Gulf War veterans.

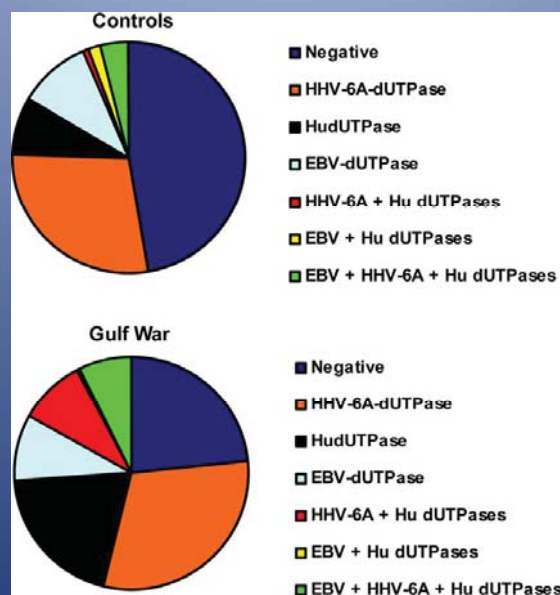
## In what ways might an infection play a role in GWI

- Triggering infections inducing downstream events
- Persistent infections
- Recurrent infections
- **Reactivation of latent infections**
- Microbiome shifts and molecular mimicry inducing autoimmunity

## Novel Properties of EBV- and HHV-6A- Encoded Deoxyuridine Triphosphate Nucleotidohydrolase (dUTPase) Proteins

- New class of pathogen-associated molecular pattern (PAMP) proteins with novel functions in innate and adaptive immunity (Ariza et al., 2009, 2013, 2014).
- Activate Toll-like receptor 2 (TLR2) signaling leading to the secretion of Th1/Th17 pro-inflammatory cytokines.
- Alter the expression of several genes implicated in the regulation of cognitive behavior, motor control, depression as well as T-cell function.
- Immunomodulatory functions may contribute to the pathophysiology of diseases caused by these viruses.

## Ab Positivity to HHV-6A, EBV and Human Encoded dUTPases by ELISA in a Cohort of Gulf War and Control Subjects



## Collaborators

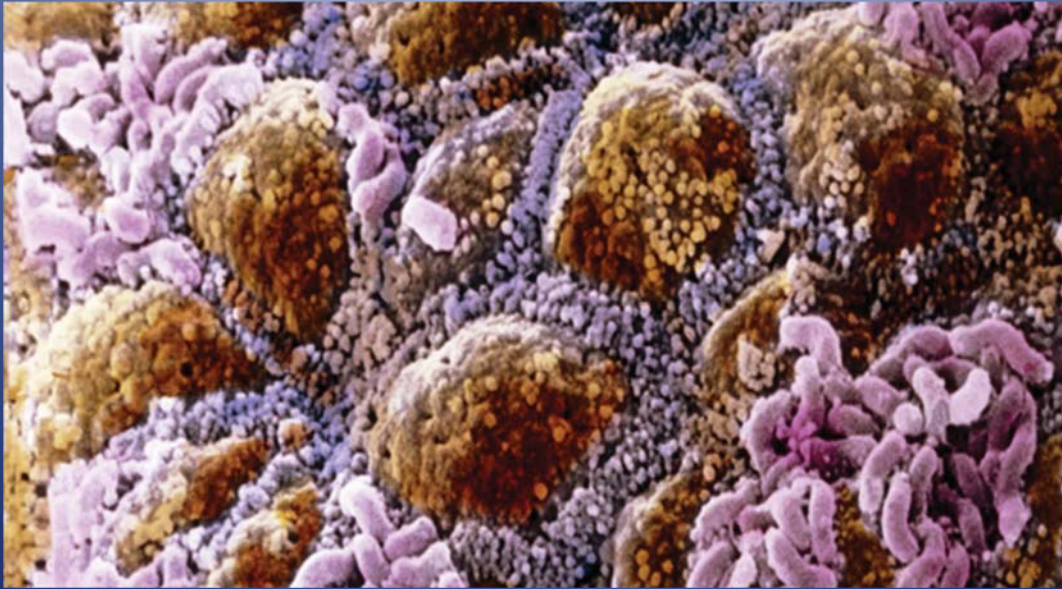
### The Ohio State University School of Medicine

- Marshall Williams, PhD
- Maria Eugenia Ariza, PhD
- Ron Glaser, PhD

### In what ways might an infection play a role in GWI

- Triggering infections inducing downstream events
- Persistent infections
- Recurrent infections
- Reactivation of latent infections
- Microbiome shifts and molecular mimicry inducing autoimmunity

## Gut microbiome



## Microbiome and health: an emerging field

- The gut contains microorganisms that share a structural similarity with the neuropeptides involved in regulating behavior, mood, and emotion—a phenomenon known as molecular mimicry, which can induce antigen specific responses that go on to target specific tissues.
- Gut flora can impact systemic and brain physiology – animal studies show the microbiome inducing neuroinflammatory states
- Gender differences in the microbiome influence systemic inflammation
- A single course of antibiotics can significantly alter the microbiome with only partial recovery up to 4 years after treatment

## Biomarkers: Future directions

- New methods force us to relook at “old” ideas with more powerful tools
- Whole genome sequencing is a powerful tool as are large platforms for autoimmune surveys, pathogen discovery etc
- When combined with neuroimaging tools, comprehensive clinical assessments and utilizing computational biology approaches to handle “big data” we have the tools in hand now to answer the complex questions we face in this field.

## RAC GWVI Recommendations

Specific RAC-GWVI recommendations include:

**“Identification of objective measures that distinguish veterans with Gulf War illness from healthy veterans.**

The Committee places a high priority on identification of biological markers for Gulf War illness and measurable differences between groups of symptomatic and healthy Gulf War veterans.

In light of findings from current and ongoing studies describing associations between Gulf War illness and neurological, immune, endocrine, genetic, and biochemical alterations, the Committee recommends the following research:

## RAC GWVI Recommendations 2

### Specific RAC-GWVI recommendations include:

Studies that utilize state-of-the-art neuroimaging technologies to characterize aspects of brain structure and function that may distinguish veterans with Gulf War illness, including illness or exposure subgroups, from healthy Gulf War veterans.

Comprehensive evaluation of autonomic nervous system function associated with Gulf War illness, as well as illness and exposure subgroups.

Research that investigates biological and genetic variability potentially linked to differences in vulnerability to Gulf War exposures, including studies that evaluate associations between Gulf War illness and genetic polymorphisms and activity levels of enzymes associated with uptake and metabolism of neurotoxic exposures.

Studies that evaluate alterations in central proinflammatory and inflammatory processes in Gulf War veterans affected by Gulf War illness.

## RAC GWVI Recommendations 3

### Specific RAC-GWVI recommendations include:

Comprehensive evaluation of immune parameters associated with Gulf War illness, including parameters that may differ among illness and/or exposure subgroups.

Comprehensive evaluation of hypothalamic-pituitary-adrenal axis and other neuroendocrine parameters in association with Gulf War illness, including parameters that may differ among illness and/or exposure subgroups.

Studies that determine the extent to which other physiological characteristics that distinguish CFS, FM, and MCS patients from healthy controls are also associated with Gulf War illness.

Studies that utilize new technologies (proteomic, lipidomic, genomic, and metabolomic methods) capable of identifying unique molecular characteristics of Gulf War illness, and of illness and exposure subgroups.

# Thank You



# Thank You



---

**United States General Accounting Office**

---

**GAO**

Testimony

Before the Subcommittee on National Security,  
Emerging Threats, and International Relations,  
Committee on Government Reform, House of  
Representatives

---

For Release on Delivery  
Expected at 1:00 p.m. EDT  
Tuesday, June 1, 2004

---

**GULF WAR ILLNESSES**

**DOD's Conclusions About  
U.S. Troops' Exposure  
Cannot Be Adequately  
Supported**

Statement of Keith Rhodes, Chief Technologist  
Center for Technology and Engineering, Applied Research  
and Methods