Research Advisory Committee on Gulf War Veterans' Illnesses

February 23-24, 2004 Committee Meeting Minutes

U.S. Department of Veterans Affairs
811 Vermont Ave, Room 819
Washington, D.C.

DEPARTMENT of VETERANS AFFAIRS

Research Advisory Committee on Gulf War Veterans' Illnesses
VA Eastern Kansas Healthcare System (T-GW)
2200 S.W. Gage Blvd. Topeka, KS 66622

I hereby certify the following minutes as being an accurate record of what transpired at the February 23-24, 2004, meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses.

_____

/signed/
James H. Binns,
Chairman
Research Advisory Committee on Gulf War Veterans' Illnesses

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Attendance Record

Members of the Committee in Attendance
James H. Binns, Chairman
Nicola Cherry
Beatrice Golomb
Joel Graves
Robert W. Haley
Marguerite Knox
William J. Meggs
Pierre J. Pellier
Steve Robinson
Steve Smithson
Lea Steele

Consultant to the Committee
Jack Melling

Committee Staff
Laura Palmer

Guest Speakers
John Concato
Roger Kaplan
Preeti Hans
Joe Gough
Alan Magill
Ya Fang Liu
Mohan Sopori
John Ottenweller
Mark Peakman
Al Marshall
Terry Pellmar
Johnnye Lewis
Melissa McDiarmid
Sam Donta

Abbreviations

AChE	Acetylcholinesterase
AFRRI	Armed Forces Radiobiology Research Institute
BLRD	Biomedical Laboratory Research and Development service (VA)
CFS	Chronic fatigue syndrome
CSRD	Clinical Science Research and Development service (VA)
CRADO	Chief Research and Development Officer (VA)
DHI	Deployment Health Initiative
DoD	Department of Defense
GAO	General Accounting Office
GWI	Gulf War illness
NSF	National Science Foundation
OEF	Operation Enduring Freedom
OIF	Operation Iraqi Freedom
ORD	Office of Research and Development (VA)
PB	Pyridostigmine bromide
PI	Principal investigator
VA	U.S. Department of Veterans Affairs

Meeting Agenda

Monday, February 23, 2004

8:30 – 8:45	**Welcome, introductions, and opening remarks**	Mr. Jim Binns
8:45 – 9:15	**Update on ongoing VA Gulf War illness research projects** *Dr. Concato, director of the Clinical Epidemiology Unit at West Haven VAMC, will provide an update on VA's study investigating levels of a mutant form of acetylcholinesterase (AChE-R) in ill Gulf veterans. Mr. Kaplan will report on other VA Gulf War illness research projects of interest.*	Dr. John Concato Mr. Roger Kaplan
9:15 – 9:45	**Research funded under the Deployment Health Initiative** *Ms. Hans is staff assistant for Gulf War illnesses in VA's Office of Research and Development. She will update the Committee on proposals received and projects funded under VA's Deployment Health and Cooperative Studies funding announcements.*	Ms. Preeti Hans
9:45 – 10:15	**Overview of VA administration of Gulf War illness research proposals, review, and funding** *Mr. Gough is Acting Director of Administration in VA's Office of Research and Development. He will discuss how Gulf War illness research proposals are scientifically reviewed, funded, and prioritized, and provide additional information on the $20 million Deployment Health funding initiative.*	Mr. Joe Gough
10:15 – 10:30	***Break***	
10:30 – 11:00	**Discussion**	
11:00 – 12:00	**Leishmaniasis in veterans of Desert Storm and Iraqi Freedom** *Dr. Magill is Science Director at the Walter Reed Army Institute of Research. He will provide an overview of detection and assessment of leishmania infection in Gulf War veterans, and preliminary information regarding leishmania infection in military personnel serving in the current conflict in Iraq.*	Dr. Alan Magill
12:00 – 1:00	***Lunch***	

1:00 – 1:45	**Role of stress-activated kinase in the pathogenesis of Gulf War Syndrome** *Dr. Liu is an Assistant Research Professor of Pharmacology at Boston University. She will discuss her research on the induction of mixed lineage kinases and neuroinflammation by stress, vaccines, and pyridostigmine bromide, and the potential use of kinase inhibitors to prevent or treat the adverse consequences.*	Dr. Ya Fang Liu
1:45 – 2:30	**Immunotoxicity of low-dose sarin and silica inhalation** *Dr. Sopori is Senior Scientist and Director of the Immunology Program at Lovelace Respiratory Research Institute. He will present results of his studies investigating immunological effects of inhaled exposure to sarin, sand, and cholinergic agents such as pyridostigmine bromide.*	Dr. Mohan Sopori
2:30 – 2:45	***Break***	
2:45 – 3:30	**East Orange VAMC research on immune parameters in ill Gulf War veterans** *Dr. Ottenweller is a Professor of Neurosciences at the University of Medicine and Dentistry of New Jersey. He will discuss studies at the East Orange VA Center for Environmental Hazards Research on lymphocyte subpopulations and cytokine levels in symptomatic and healthy Gulf veterans.*	Dr. John Ottenweller
3:30 – 4:30	**Immune activation and Th1/Th2 cytokine balance in Gulf War-related illnesses** *Dr. Peakman is with the Department of Immunobiology in the School of Medicine at King's College in London. He will present results of a study of cellular immune activation associated with multisymptom illness in British Gulf War veterans.*	Dr. Mark Peakman
4:30 – 5:00	**Discussion**	
5:00 – 5:30	**Public Comment**	
5:30	**Adjourn for the day**	

Tuesday, February 24, 2004

8:00 – 9:00	**Overview of potential health consequences of depleted uranium use** *Mr. Marshall is with Sandia National Laboratories in Albuquerque. He will provide an overview of possible concerns related to radiological and chemical effects of DU exposures in Gulf War veterans.*	Mr. Al Marshall
9:00 – 10:00	**Research on the effects of depleted uranium from the Armed Forces Radiobiology Research Institute** *Dr. Pellmar will report on the results of multiple studies conducted by investigators at the Department of Defense's Armed Forces Radiobiology Research Institute assessing the biological effects of implanted DU fragments in animals.*	Dr. Terry Pellmar
10:00 – 10:15	***Break***	
10:15 – 10:45	**Studies of the uptake and neurological effects of inhaled uranium** *Dr. Lewis is Director of the Community Environmental Health Program at the University of New Mexico. She will report on her research on the potential for inhaled uranium to enter the brain through the nose and cause neurological damage.*	Dr. Johnnye Lewis
10:45 – 11:30	**Surveillance of veterans exposed to depleted uranium during the Gulf War** *Dr. McDiarmid directs VA's Depleted Uranium Program at the Baltimore VAMC. She will present an overview of the results of the program's ongoing clinical surveillance of Gulf War veterans who have embedded DU fragments, DU-contaminated wounds, and significant inhalational exposure to DU.*	Dr. Melissa McDiarmid
11:30 – 12:00	**Discussion**	
12:00 – 1:00	***Lunch***	
1:00 – 1:30	**Overview of research on infectious diseases in Gulf War veterans**	Dr. Lea Steele
1:30 – 2:15	**Findings on mycoplasma infection and treatment from VA's antibiotic treatment trial** *Dr. Donta is a retired Professor of Medicine at Boston University Medical School and principal investigator of VA's antibiotic treatment trial. He will present findings from the study and discuss their implications with respect to the role of mycoplasma infection and antibiotic treatment in Gulf War veterans' illnesses*	Dr. Sam Donta
2:15 – 3:00	**Update on recently published research**	Dr. Beatrice Golomb

3:00 – 3:30	**Committee business**
3:30 – 4:00	**Public comment**
4:00	**Adjourn**

Welcome, introductions, and opening remarks

Mr. James H. Binns, Jr., Chairman

Chairman James Binns called the meeting to order at 8:30 a.m. He stated that he had high expectations for the Committee for three reasons:

1. Significant scientific progress made in last eighteen months by the people speaking at this and previous Committee meetings.

2. Progress by the Committee and its new staff organization, which was reflected in the quality of this meeting and preparation that had gone into it.

3. Changes taking place over the past two years at the Department of Veterans Affairs (VA). Chairman Binns cited the enormous support from Secretary Principi. He noted that, due to the Secretary's initiative, up to 20 million dollars had been committed to research for Gulf War illnesses. He also noted the development of a pattern, such as the work done with Dr. Soreq, where the Committee worked with VA's Office of Research and Development (ORD) to hand off an idea and see VA run with it.

Chairman Binns noted the loss of Dr. Nelda Wray as a champion within VA for Gulf War veterans. He expressed the Committee's gratitude for the contributions she made and extended well wishes in her future endeavors. He stated that the progress she began was going to continue, through new officials, like Dr. Jonathan Perlin, Deputy Under Secretary for Health and Acting Chief Research and Development Officer (CRADO). Chairman Binns read a message from Dr. Perlin, which was as follows:

"I would like you to know that Mindy (Aisen) and I emphatically support research into the illnesses of Gulf War veterans. I would ask for your help in conveying this message to the Committee as it is our desire to continue the tradition of collaboration, mutual respect and shared purpose."

Chairman Binns welcomed a joint effort between ORD and the Committee. He indicated that the pieces were in place, and it was time to start doing something about it. He stated that the Secretary expected it, along with 175,000 ill veterans. He stated that he believed this was a deliverable goal.

Chairman Binns asked Dr. Lea Steele, the Committee's Scientific Director, to say a few words. She introduced Ms. Laura Palmer, who joined the Committee's staff in December, 2003. She then asked Committee members to introduce themselves to the audience.

Chairman Binns introduced Dr. Mindy Aisen, Deputy Chief of Research and Development. Dr. Aisen is a Board-certified neurologist, a Fellow of the American Academy of Neurology, and President of the American Society of Neurorehabilitation, with special interests in multiple sclerosis and other neurodegenerative diseases.

Dr. Aisen gave a brief overview about the reorganization of ORD research services which was being undertaken to run a more fluid program. She stated that ORD was absolutely committed to the scientific peer review system, working to make it better by making sure the top experts in the field were reviewing grant applications with scientific rigor. She stated that ORD also was working with clinical and veteran communities to ensure research being funded was relevant and not duplicative of what other agencies are doing. She indicated that ORD actively had been recruiting a PhD scientist to oversee the Gulf War illnesses program. She stated that ORD understood that, while the causes still were not clear, people were

ill and that was ORD's obligation to find out what the etiology is, what the nature of the problem is, and what sort of treatments may be useful.

Acetylcholinesterase activity in Gulf War deployed and era veterans: February 2004 update

John Concato, MD, MS, MPH
Director of Clinical Epidemiology, West Haven VA Medical Center

Dr. John Concato gave a status update on a VA research study being conducted by a team that includes Bradley Doebbeling, MD, MSc (Indianapolis), Peter Peduzzi, Ph.D. (West Haven), Hermona Soreq, PhD (Jerusalem), Catherine Viscoli, Ph.D. (West Haven) and himself. (See Appendix A – Presentation 1.) The following three hypotheses were being tested:

1. Mood and anxiety symptoms are associated with selected blood enzyme levels

2. Deployed (vs. non-deployed) Gulf War veterans have lower capacity to increase blood acetylcholinesterase (AChE) levels

3. Veterans with (vs. without) symptoms of Gulf War Veterans Illness (GWVI) have lower capacity to increase blood AChE levels in response to challenge

Dr. Concato stated that all data and laboratory analyses had been completed. Analyses of the initial hypotheses had been conducted, with other analyses being considered at this time. He indicated that the splice AChE-R data should be available by the next Committee meeting.

Dr. Beatrice Golomb suggested that it will be important to compare veterans who meet the CDC case definition for multi-symptom illness to veterans who consider themselves healthy, that is, who don't have any of the CDC symptom criteria. She also questioned one of the study's hypotheses, stating that one would not necessarily expect to see a reduced level of AChE in ill veterans exposed to AChE inhibitors. She stated that it was important to compare AChE levels in ill Gulf veterans who report AChE exposures, as opposed to all ill veterans, regardless of exposure, as combining all veterans could dilute findings that might otherwise be significant. She was encouraged to see that future analyses would include an examination of exposures.

Dr. Steele noted that Dr. Soreq had reported, at the October 2003 Committee meeting, an increase in acetylcholinesterase in Gulf war veterans compared with the normal population. She inquired as to whether this remained true. Dr. Concato stated it did, but all characteristics of the population needed to be accounted for before making assumptions about any differences. The Heritage Family Study was not a random sample of the U.S. population. Researchers didn't have access to all variables when this study was discussed in October, 2003. Further analyses, including these variables, were on-going.

Dr. Pierre Pellier inquired as to the proportion of ill veterans with anxiety disorder in the study's sample. Dr. Golomb inquired as to whether adjustments had been made for anti-depressant medication use. Dr. Concato indicated that they had, and there was little variation in the results.

Dr. Nicola Cherry inquired as to how the researchers were conducing validity checks of the self-reported exposures. Dr. Concato indicated that his group was being cautious by looking at the Iowa study's original questions to see if the questions looked at the same exposures, time served in Gulf, etc.

Dr. Golomb inquired as to whether the researchers would be able to ask "de novo" whether the Iowa study's reported symptoms did match CDC criteria, and if not, noted this might lead to misclassification and dilution of findings. Dr. Concato stated a "de novo" review was outside of the scope of this study, but they were aware of this concern and would be addressing in future reports how they were able to match the symptoms, as best as possible, to the CDC criteria.

Dr. Robert Haley inquired as to whether Dr. Soreq had calculated the alloenzyme concentrations of paraoxase and arylestease. Dr. Concato stated he would check into this.

Dr. Concato's talk concluded.

Dr. Aisen informed the Committee that Dr. Perlin was at an international meeting in New Zealand. The focus of this meeting was to develop a tighter collaboration between the US, UK, Canada, New Zealand and Australia with regards to Gulf War issues, including health. One of the plans is to develop a database that will be span these five countries. She stated that a follow-up research meeting was tentatively planned to take place in Hawaii.

VA Gulf War Illness Research Project

Roger Kaplan
Special Assistant, Central Office, VA Research and Development

Mr. Roger Kaplan provided the Committee with an update as to the recent reorganization within ORD. Previously, there was a large Biomedical Research and Development Service, charged with laboratory research and single-site clinical trials. Now, there will be two services: Biomedical Laboratory Research and Development (BLRD) and Clinical Science Research and Development (CSRD). CSRD will oversee all clinical trials [single and multi site (previously part of the Cooperative Studies section)]. Dr. Timothy O'Leary will be the new BLRD Director. A new CSRD director will be appointed soon. Two ORD services remain unchanged: Rehabilitation Research and Development Service and Health Services Research and Development Service.

Mr. Kaplan stated that ORD was in the process of re-announcing the Gulf War research specialist position. Besides his acting duties regarding Gulf War research, Mr. Kaplan primary duties entail Congressional liaison, veterans' outreach, biosecurity, biosafety, executive intra-correspondence, and inter-governmental relations.

Mr. Kaplan gave a progress report on Dr. Mike Weiner's Department of Defense (DoD) five-year study on the effects of Gulf War illnesses on brain function and structure. (See Appendix A – Presentation 2.) This study is a continuation of Dr. Robert Haley's research with the 24th CB battalion.

During discussion, Dr. Haley indicated that Dr. Weiner would be looking at several different case definitions.

Mr. Kaplan's talk concluded.

VA'S Deployment Health Initiative

Preeti Hans, MHP
Staff Assistant, Gulf War Illnesses, Central Office, VA Research and Development

Ms. Preeti Hans gave an overview of the Gulf War illnesses-related studies that had been funded through VA's ORD services. (See Appendix A – Presentation 3.)

Chairman Binns expressed dismay that only one study had been funded under the Deployment Health Initiative (DHI) since July 1, 2003. He stated that, of the sixteen studies funded/proposed as of last fall, only four studies really related to Gulf War illnesses. He noted that some of the other studies focused on stress hypotheses, although the Committee has pointed out that such hypotheses have not proven to be useful, as a central focus, in shedding light on Gulf war illnesses (GWI).

Mr. Kaplan stated that the ORD was also disappointed that only six proposals had been received under the DHI last fall. He indicated that the ORD would be doing more during the next proposal round to encourage field researchers to develop meaningful proposals. He cited reduced budgets contributing to the problem.

Dr. Golomb asked, in general, what percentages of the proposals were focused on stress vs. organic exposures. Mr. Kaplan stated that all three Clinical Biomedical proposed studies, which were denied, dealt with stress or anxiety. He stated that looking through the research portfolios, staff had identified GWI-related studies that had not been applied for under the DHI RFP. He stated that in terms of overall deployment-related studies, just over 9 million dollars had been committed.

Dr. Aisen noted the importance of understanding how a research program works and the many different relationships with various entities to produce quality work. She stated they needed to maintain credibility by funding projects that met the rigor of peer-review scrutiny. She discussed the scoring system and the need to improve the quantity of proposals, which would lead to a better quality of proposals. She stated that ORD was sensitive to the needs of deployed veterans and had decided to take other approaches to solicit more applications that are relevant to all of these types of conditions. She indicated that they didn't want to develop a program that lumped together diseases, e.g. neurodegenerative diseases, and call these "Gulf War illness." She stated their office needed to: (1) "prime the pump"; (2) identify potential researchers and the questions that they could investigate; and (3) encourage their service on review committees.

Chairman Binns stated this was good news, because the Committee had been hearing that, because of the failure to fund previous Gulf War related proposals, field researchers were discouraged about submitting additional proposals. He stated that Dr. Wray, the former CRADO, expressed intent to create a more pro-active program and was delighted to hear this stance would continue. He stated that he knew, from experience, research proposals needed to be designed to get to the particular goal, as opposed to acceptance of random proposals from field researchers.

Dr. Haley made a suggestion; based on his observations, the lack of proposals may be due to a lack of acceptance by many VA physicians that Gulf War illness exists. He suggested that the controversy as to whether Gulf War illness was a psychological, rather than organic, condition had handicapped the field. He wondered whether researchers didn't consider this field of study because they didn't believe it to be an organic illness, or perhaps they would be handicapped for expressing interest in the subject matter. He stated that this perception needed to be overcome, perhaps with some public relations to encourage field researchers' development of unique approaches to the problem.

Fall 2002 Deployment Health Solicitation and Review Process

Joe Gough, MA
Acting Director of Administration, Central Office, VA Research and Development

Mr. Joe Gough gave an overview of the Fall 2002 Deployment Health Initiative solicitation and review process. (See Appendix A – Presentation 4.) Mr. Gough noted that this solicitation remained open for additional proposals.

Dr. Steele asked whether there was a panel of people to review these applications with Gulf War illness expertise, or were the proposals assigned to boards that were not particularly familiar with Gulf War illnesses. She stated that there seemed to be no real prioritization given to Gulf War related proposals and that 20 million dollars had not been specifically set aside for Gulf War research.

Dr. Gough stated that Dr. Steele was correct; there was no specific Gulf War related illness review board. There were separate review boards for the Biomedical Laboratory and Clinical Sciences services. The Biomedical Laboratory Service, alone, has 21 sub-specialty specific boards.

Dr Aisen stated that there was a strong tradition within the VA research community. She indicated that Dr. Wray had provoked much controversy when she tried to introduce the notion of accountability and productivity to the review process. In retrospect, Dr. Aisen stated that these concepts probably weren't introduced gradually enough. She stated that the concepts needed to be introduced very openly and objectively. She stated that didn't mean ORD always says the recommendations of the advisory review panels are just that- recommendations from an *advisory* review panel. However, she stated that there was always going to be situation where the CRADO, once within the range of funded vs. unfundable proposals, had discretion in making final decisions. She stated that former Deputy Secretary Mackay had indicated that up to 20 million, not at least 20 million, dollars would be spent for Gulf War related illness. She stated that ORD had to be responsible with the funding decisions, keeping in mind that there are many of other important veteran medical concerns.

Chairman Binns asked if there was another avenue for the Committee and ORD staff to work together to develop a "top-down" research plan, rather than relying on general solicitation announcements. He believed that if field researchers knew there was money available, they might develop a proposal that would score high under peer review without sacrificing scientific quality, and achieve the ultimate goals relating to Gulf War illnesses.

Dr. Aisen noted that collaboration with advisory committees, like the Soreq collaboration, was fairly unusual within ORD. She stated that ORD was working towards building a more clinically-orientated research program. This process couldn't be sudden, and they were trying to encourage development of more clinical proposals in order to justify funding more of them. She wondered if there was a way to identify a certain set of treatment questions for Gulf War illnesses. She didn't want to single out researchers and ask them to write proposals because without competition, this might compromise the overall quality of the work.

Chairman Binns stated the Committee was trying to determine what was needed to stimulate VA GWI research. He stated that the veteran community saw the 20 million dollar funding initiative as a huge step forward, and that the science seemed to be there. Dr. Aisen agreed that genuine Gulf War research would grow in the future.

Mr. Joel Graves expressed his exasperation with ORD and its approach to the 20 million dollar funding initiative. He understood the Secretary's mandate made Gulf War illnesses research a top funding priority. If a proposal didn't meet scientific criteria, ORD would move on to other proposals. While the other proposal may be attractive and great science, Mr. Graves felt, if a proposal didn't met the primary criteria, its funding would be a misuse of research monies. Mr. Graves also believed that because the funding initiative was in response to the Committee and its recommendations, it was imperative that a representative(s) from the Committee be involved in the decision-making process.

Mr. Steve Robinson stated it was his understanding that the Committee, by Congressional charter, had federal oversight into anything and everything that had to do with Gulf War illnesses and federal Gulf War research, especially within VA. He asked whether a relationship between ORD and the Committee existed where the Committee was being made aware of what proposals were being submitted, and what, in terms of the Committee's concerns, was being done. He stated that discussions with the former CRADO had found an openness to create mechanisms whereby the Committee was in the loop as to what proposals were being received, what proposals were being funded, and where ORD stood in terms of approval of these proposals. He stated that he understood ORD's confidentiality concerns, but noted that there were scientific experts on the Committee that dealt with these concerns every day.

Dr. Aisen indicated that she thought there were mechanisms to create such a relationship. She stated that it wasn't unique for an advisory committee to want more of a role in the decision-making process, and that additional input was useful to the scientists.

Mr. Robinson stated he didn't expect to hold the new CRADO to the former CRADO's promises, but noted that this Committee had to answer to veterans. If the Committee didn't know what was going on, they wouldn't be able to respond to veterans' questions and concerns. He stated that even knowledge of proposal themes would help the Committee's understanding of funding statuses.

Dr. Aisen believed that the Committee would be pleasantly surprised with the proactive approach that ORD would be taking. She also believed there were still questions within the scientific community as to whether this was a syndrome that represented several problems or not. She acknowledged that the manifestations were different, but there wasn't enough scientific knowledge to say this was all basal ganglia regeneration, chronic infection, etc. Because of this uncertainty, it was difficult for ORD to say a proposal did or didn't relate to Gulf War illness.

Dr. Golomb stated that there was an acceptance that there are illnesses and there may be different exposures/factors. She expressed her concern that a core group of veteran advocates be given a confidential role into research.

Mr. Robinson stated that one of most troubling aspects of this situation is the Committee's lack of knowledge of current research funding. He told a story about being asked by a veteran about the Committee's accomplishments since it was appointed. He was able to list several things that had been done, but he also had to acknowledge what the Committee couldn't do because it hadn't had a view of the situation.

Chairman Binns indicated he had a similar interaction. He told a story about meeting with former Deputy Secretary Mackay after the June 2003 Committee meeting. Mr. Mackay asked a similar question: "How's the research coming? Are you getting some good research?" Chairman Binns had to respond that these were good questions, but he wasn't able to answer them. As such, former Deputy Secretary Mackay tasked Dr. Wray, former CRADO, to solve this problem. Chairman Binns stated that the Committee was glad to hear that ORD was not happy with the DHI solicitation process outcome, with respect to Gulf War

related research. He hoped that a solution could be found, taking into account ORD's confidentiality concerns, which would provide a closer meshing between the Committee and ORD, as well as a larger list of high quality proposals for funding that cover all development issues in the multiple illness consideration.

Dr. Aisen stated that ORD was working with field researchers addressing similar concerns, e.g., how review committees were chosen, how policy funding decisions were being made, etc. She reiterated ORD's openness to achieving more quality research.

Chairman Binns noted that Dr. John Ottenweller, a researcher from the East Orange, NJ, VA Medical Center, was in the audience. Chairman Binns asked Dr. Ottenweller if he could provide perspective as a VA researcher on the situation.

Dr. Ottenweller indicated that he could make two comments as to the ongoing discussion. First, he stated that field researchers had information from ORD that there was, in fact, no set aside funds or special review panel for the DHI. Thus, they were told there was no advantage to submitting a LOI under it. He stated there were researchers in his group that submitted half a dozen proposals relating to Gulf War illness that weren't included under the DHI. Dr. Aisen inquired as to the time frame for this information. Dr. Ottenweller stated it would have been in 2001, 2002 and early 2003. Dr. Aisen was surprised and requested more specific information, which Dr. Ottenweller offered to provide later.

Second, Dr. Ottenweller made a general comment about the "disconnect" between policy makers and the review board. He believed most people thought reviewers have criteria given to them to prioritize proposals but this wasn't always the case. From his experience on a review board, without an established list of administration policies, the individual reviewer's personal priorities may control their decisions.

Dr. Golomb noted there was scientific support for this. She stated that the NSF conducted a study, where they gave a set of proposals to independent review committees, and then compared the reviewers' scores. She stated that they found the concordance was a little better than chance. She agreed that, if you set aside factors on which to score things, an individual's perception of importance would vary.

Dr. Aisen noted that peer review was still an important aspect of funding good science. She did acknowledge that, while peer review was divine, the reviewers were human. She stated this just means "we" need to figure out how to improve the process. Dr. Aisen reiterated ORD's openness to improving the proposal process, making the portfolio more relevant, making sure the investigators care about veterans' concerns.

Chairman Binns expressed his appreciation for Dr. Aisen's interest in working with the Committee on these concerns. He noted that there was a psychological issue to the initiative being based on fiscal '04 funding. Being this was the year in which there finally was a commitment by VA leadership to address Gulf War illnesses, the Committee would like to capitalize on this momentum, and would be available in any way that would help.

Chairman Binns thanked ORD staff for speaking that morning.

The meeting adjourned at 11:00 a.m. for a ten-minute break.

The meeting reconvened at 11:10 a.m.

Leishmaniasis in veterans of Desert Storm and Iraqi Freedom

Alan J. Magill, MD, FACP
Science Director, Water Reed Army Institute of Research

Dr. Alan Magill provided an overview of the pathology, diagnosis and treatment of leishmaniasis. (See Appendix A – Presentation 5.) He discussed the diagnosed cases in Desert Storm/Desert Shield and Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) veterans. He indicated that recent discussion about this disease, in connection with Desert Storm/Desert Shield veterans, was non-existent and welcomed a chance to revisit this matter, with a ten-year perspective.

With respect to OIF, Dr. Magill stated roughly 500 cases of cutaneous leishmaniasis (*L. major*) had been diagnosed. He indicated these cases were relatively easy to diagnosis (typical lesions) and were responding to therapy. He estimated that, based on what was being seen in the rotations coming out of Iraq, there would be over 1,000 cases, making this the biggest infectious disease problem in the current conflict. He did note that two soldiers who were stationed in Afghanistan had been diagnosed with visceral leishmaniasis the previous week.

Following Dr. Magill's presentation, Dr. Haley asked whether subclinical leishmaniasis might be a potential cause of the illnesses experienced by a portion of the ill Desert Shield/Desert Storm veterans. Dr. Haley wondered whether there might be a large group that may have potentially been infected, say, up to 20,000. Dr. Magill indicated that didn't fit with his observations of this disease in Desert Storm veterans.

Dr. Haley inquired as to what reliable tests were available. Dr. Magill stated that serology, with the current available antigens, was not helpful. From a basic pathogenesis point of view, he stated clinicians and researchers shouldn't spend a lot of time with this test. He would focus on CMI (cell-mediated response) testing for antigens that could be reliably detected.

Dr. Haley asked whether a skin test would be a reasonable way to test the hypothesis, in an epidemiological study, that leishmania was a cause of some Gulf War veterans' illnesses. Dr. Magill stated it would be helpful, but there currently was no reliable skin test available. He said that DoD had been developing such a test, but had halted the program in September, 2003. Dr. Magill stated that a cell-mediated immune response (CMI) test was a more viable solution. He noted there were on-going discussions to develop a CMI assay for leishmania, which was similar to the QuantiFERON assay for TB. Dr. Magill thought that, once a CMI test had been developed, it would make sense to go back and examine whether more Desert Shield/Desert Storm veterans were exposed to/infected with leishmania.

Dr. Haley noted, that in developing countries, treatment was given based upon symptoms, rather than clinical test confirmation, because of cost concerns. He asked whether it be reasonable to do a clinical trial, using one of the leishmaniasis treatments, with Desert Shield/Desert Storm veterans meeting the case definition of GWI. He stated this was a difficult call, but noted that some of these veterans' lives have been ruined. Dr. Magill stated that this option had been discussed, but that the side effects of the available drugs were potentially significant. He also expressed concern about controlling for placebo effects.

Dr. Mohan Sopori asked if research was being done to identify an immunological marker(s) to distinguish between various levels of leishmaniasis. Dr. Magill indicated that most of the immunological research on leishmania was general, using the parasite as a model organism. He stated that clinical outcomes were not an objective of most of this research.

Steve Robinson asked if all 500 OIF leishmaniasis cases had been evacuated from theater. Dr. Magill indicated that about 200-250 cases had been evacuated because they had multiple lesions.

Mr. Robinson asked what was the primary defense to leishmaniasis. Dr. Magill indicated that personal protection techniques (pesticides, mosquito netting, etc.) were used to protect soldiers from leishmania infection. Mr. Robinson asked if the anti-malarial drug had any effect on leishmania. Dr. Magill indicated that it did not.

Chairman Binns asked Dr. Magill for his thoughts and recommendations as to future leishmania research that would make sense for the ill veterans of the first Gulf War.

Dr. Magill indicated that he could envision a few hundred veterans having been infected, but not diagnosed. To develop a research approach with respect to these veterans, he indicated that he would assemble a multi-disciplinary team (immunologists, epidemiologists, neuropsychologists, etc.), and then examine veterans from units that had been in high-risk exposure conditions.

Dr. Cherry asked whether there was a treatment if it was determined these veterans had been infected. Dr. Magill indicated that he was not confident this would be the case, just as there was no treatment for chronic fatigue syndrome.

The meeting adjourned at 12:15 p.m. for lunch.

The meeting reconvened at 1:20 p.m.

Assessment of a role of stress-activated kinases in the pathogenesis of Gulf War Syndrome

Ya Fang Liu, MD, PhD
Department of Pharmacology, Boston University School of Medicine

Dr. Ya Fang Liu gave a presentation about the role of stress-activated kinases in the pathogenesis of Gulf War illnesses. (See Appendix A – Presentation 6.) She examined the effect of stress, vaccines and pyridostigmine bromide (PB) on stress-activated kinase activity. During her talk, Dr. Haley asked about the use of adjuvant in the vaccination in-vivo portion of her study. Dr. Liu indicated that TRH had been used. Discussion occurred about whether the tested substance was an "adjuvant" or simple protein. Dr. Liu stated that these studies were preliminary, and that the results were based upon one-year's worth of work. She stated that she hoped this study would bring some light to the research area and provide a treatment for ill Gulf War veterans.

Upon conclusion of her talk, Chairman Binns asked Dr. Liu to address possible treatment studies in light of her findings with stress-activated kinases. Dr. Liu stated that animal studies needed to be conducted, testing the effect of CEP-1347. She stated that verification, through point mutation studies, needed to be done to verify the drug's ability to inhibit stress kinase activation.

Dr. William Meggs commented that Dr. Liu's work was exciting, not only because it might lead to a treatment, but also lead to a diagnostic test. Dr. Liu stated that kinase levels could only be measure indirectly (via cytokine levels) at this time. She stated that direct kinase level tests haven't been developed yet.

Discussion occurred between Dr. Liu and Dr. Sopori about clinical trials and various levels of kinases.

Immunotoxicity of low-dose sarin and silica inhalation

Mohan Sopori, PhD
Senior Scientist and Director, Immunology Program, Lovelace Respiratory Research Institute

Dr. Mohan Sopori gave a presentation regarding his preliminary findings as to immunosuppressive effects of sarin and silica in animal (rat) studies. (See Appendix A – Presentation 7.) Dr Sopori indicated he and his colleagues had begun doing these studies in conjunction with their studies of the effects of nicotine, a cholinergic agent, on the human system. During the presentation, Dr. Haley asked as to the time frame in which sarin suppression of cortisone was observed. Dr. Sopori stated the period of time was 5 days. However, he stated his team hoped to do further experiments to determine exactly how long this suppression occurs.

Following Dr. Sopori's talk, Chairman Binns asked that discussion be postponed until the discussion period scheduled later in the afternoon.

The meeting adjourned at 3:10 p.m. for a ten-minute break.

The meeting reconvened at 3:20 p.m.

Immune dysregulation in Gulf veterans with CFS and its relationship with cognitive function and functional status

John Ottenweller, MD
Professor, Neurosciences Department, University of Medicine & Dentistry of New Jersey and
Senior member, New Jersey Environmental Hazards Research Center and War-related Illness and Injury Study Center, West Orange, NJ VA Medical Center

Dr. John Ottenweller spoke about his group's evaluation of Desert Shield/Desert Storm veterans' cytokine and cortisol levels, and their relationship to the veterans' cognitive and functional status. (See Appendix A – Presentation 8.)

Following the talk, Dr. Jack Melling asked, in light of their data, if Dr. Ottenweller thought a treatment to address the Th1/Th2 balance, was worth considering. Dr. Ottenweller indicated that it was premature to say either way at this time. He believed more study/data was needed to confirm this imbalance is occurring, and avoid possibly making that imbalance worse.

In response to Dr. Magill's concerns about sampling, Dr. Ottenweller indicated that preliminary data also showed that cortisol levels in the Desert Shield/Desert Storm veterans sampled were more variable than cytokine levels. With this higher variability, duty type (guard/reserve vs. active) became a significant factor. Those veterans who had been deployed in guard/reserve capacities had significantly lower cortisol levels seven to ten hears after deployment than the other groups. Dr. Ottenweller also noted that as veterans' fatigue levels increased, cortisol levels lowered. Dr. Haley asked if this might be age-related, considering the guard/reserve unit troops were typically older. Dr. Ottenweller stated that they had looked at this, and found no relationship between age and cortisol levels.

Dr. Golomb asked if Dr. Ottenweller's group had considered that the observed increased mRNA levels might be due to increase turnover, perhaps because of increased immune activation. Dr. Ottenweller indicated that they had, and that further study was needed to answer this question.

Dr. Melling commented that he found Dr. Ottenweller's data extremely interesting in light of a conversation with a colleague about the use of anthrax vaccine in the first Gulf conflict. His colleague had mentioned that reserve forces were targeted for anthrax vaccination because these forces were considered to be at a high risk for biological attack. His colleague indicated that the prevailing thought was the Iraqis would follow the Soviet bioweapon attack model.

Chairman Binns thanked Dr. Ottenweller.

Immune activation and Th1/Th2 cytokine balance in Gulf War-related illnesses

Mark Peakman, MBBS,BSc,PhD,FRCPath
Department of Immunology, King's College School of Medicine and Dentistry, London

Dr. Peakman spoke about his group's immunological findings, or more specifically cytokine balance findings, in ill Gulf War veterans. (See Appendix A – Presentation 9.) To better understand his power point slides, Dr. Peakman provided the following abbreviation definitions: sBEV = sick Bosnia era veterans; wGWV = well Gulf War veterans; and sGWV = sick Gulf War veterans. He also noted that the green panels indicated abnormal (or significant) findings, e.g. elevation of IL-4, IL-γ, IL-2, etc.

During the talk (at Slide 12), Dr. Haley noted that he had concerns about the GWI case definition being used in this and other studies. He noted that the case definition used was non-specific, and as such might lessen the significance of the results. He felt this was the Achilles heel of this research field. Dr. Haley enquired if they might be able to review the original Wessley data set and establish a more precise GWI case definition. Then, they could re-examine these veterans (estimated about 150). This might provide insight or generate new hypotheses, as often the outliers are the key to what is occurring. Dr. Peakman acknowledged the criticism, and noted the definition became "locked" in 1996. He stated he was struck by how their results paralleled those of Dr. Ottenweller's study, i.e., a Th2 response that was not robust, and a change in IL-10 production.

During Dr. Peakman's discussion of Slide 13, Mr. Robinson asked if Dr. Peakman's group had seen an increase in eosinophils at any stage, and if so, were the veterans who received multiple vaccines more likely to have higher levels of eosinophils. He noted that last summer; several soldiers came down with a mysterious pneumonia, and evidence of high eosinophil levels. Dr. Peakman indicated that he didn't have the data at that time.

During Dr. Peakman's discussion of Slide 39, Dr. Golomb commented that single-administration of vaccines was not typical. Dr. Peakman noted that dendritic cells don't have memory capabilities. Dr. Golomb acknowledged this, but suggested that it would be interesting to look at this in an in-vivo model to examine the expression of the different cytokines. Dr. Peakman agreed that this would be possible in a nicely controlled model.

Following the talk, Dr. Haley indicated that the most conclusive finding was with regard to IL-10 production, and wondered what it might mean. Dr. Peakman stated that he was investigating three or four possible immunological mechanisms, but needed to dissect the question more clearly with clinical studies. Dr. Haley submitted for discussion whether, in light of Dr. Sopori's animal data, there might be a primary neurological mechanism having this effect on the immune system. Dr. Peakman noted that this seemed to be related specifically to Gulf War illnesses, as it wasn't observed in chronic fatigue syndrome (CFS) patients.

Chairman Binns reopened Dr. Sopori's presentation for discussion. Dr. Haley noted that the immunologic abnormality reported by Dr. Sopori's team seemed to be associated with the sympathetic nervous system, and asked if Dr. Sopori saw a connection with IL-10. Discussion followed.

Mr. Graves asked, in light of the day's discussion about cytokines and Th1/Th2 levels, what types of treatments were available to balance these cytokines. Dr. Sopori indicated that, in asthma patients, researchers have been trying to raise Th1 levels using CpG immunization. Dr. Magill noted that IL-10 excess was a common feature in visceral leishmaniasis. As such, it was noted that a low-level/residual, undetected leishmania infection might be the cause of the reported IL-10 levels in ill Gulf War veterans. Dr. Magill noted that CpG therapies are not FDA licensed at this point, but indicated that CpG therapy for primary leishmaniasis immune therapy was being studied.

Chairman Binns asked each speaker if they would make recommendation as to what they envisioned being the most worthwhile topics to pursue in future research related to Gulf War illness.

Dr. Sopori indicated that more research was needed into whether these veterans were experiencing a subclinical/occult type of infection. Discussion occurred about leishmania rates in current deployed troops and the Iraqi population.

Dr. Liu stressed her concern that, considering the period of time since the Gulf War, treatments needed to be a focal point. She expressed excitement about the possibilities of a new Parkinson's drug in clinical trials, as well as a botanical treatment that she was researching. Dr. Golomb agreed that treatments were very important, but stated mechanism answers needed to be pursued. Dr. Golomb noted that Gulf War veterans have something different than other veterans, and the other possibilities shouldn't be discounted. Dr. Pellier expressed caution about raising veterans' hopes and making undeliverable treatment promises. He noted that there were lots of diseases, e.g., irritable bowel syndrome, stroke, etc., where little was still known. He noted that when studying a syndrome, researchers struggle to determine the underlying abnormality because of its complexity. He suggested that more symptomatic approaches might be advisable at this time, due to a lack of understanding about the syndrome's physiology.

Dr. Ottenweller expressed a concern about having baseline information (data and blood samples) for the OIF/OEF soldiers. He stated concerns had arisen in their research about whether observed/reported conditions were pre-existing or war-related. He stated that his group had tried to propose three or four blood studies, but hadn't received cooperation from DoD or VA. Mr. Robinson expressed concern about the failure to collect this information again. He indicated that many groups were asking for the data, but it was proving to be difficult. Dr. Ottenweller mentioned the January 21, 2004, OIF roster analysis, but stated it didn't contain a GWI category.

Dr. Sopori stated that the key was to figure out how the central nervous system responds in ill Gulf War veterans, and figure out why these responses trigger these types of reactions. Dr. Ottenweller indicated that his group was starting to look at this question by examining cytokine levels in CFS patient cerebral spinal fluid. Dr. Liu suggested examination of GWV brain samples might provide insight as well.
Chairman Binns thanked all of the speakers. He noted that, by seeing the complexities and differences among their hypotheses, even though inter-related, researchers could begin to fill in the pieces. He stated that, by looking at all these hypotheses together, maybe a treatment would become more evident.

Public comment – Day 1

Chairman Binns opened the floor to public comment.

Mr. Dan Fahey addressed the Committee and thanked them for reviewing the issue of depleted uranium (DU) the following day. [Copies of Mr. Fahey's written statement were distributed for the Committee's review. (See Appendix B - Public Submission 1.)] Mr. Fahey provided the Committee with an overview of problems he had found with DoD/VA study assessments of DU's effects on Gulf War veterans. He made two recommendations to the Committee: (1) the VA's DU study should be expanded to include the approximately 900 veterans identified by DoD as having Level I or II exposures during the 1991 war; and (2) due to concerns about data filtering/lack of disclosure in the current DU study, new VA program leadership enlisted to oversee the expanded study.

The meeting adjourned for the day at 6:00 p.m.

The meeting reconvened the following day, February 24, 2004, at 8:10 a.m.

Preliminary assessment of DU munitions health effects

Al Marshall
National Security Studies Department, Sandia National Laboratories

Mr. Al Marshall gave an overview of the results of the findings of his project investigating possible risks related to DU exposure, particularly in Gulf War veterans. (See Appendix A – Presentation 10.) One slide (#15) correction was noted, in that the arrow showing DU's pathway to the blood stream should have been shown from the small intestine, not the large intestine.

Following the talk, Dr. Haley asked about the amount of DU found in the brain. Mr. Marshall stated that DU definitely enters the brain through the blood and that it appears to happen rapidly. He stated that, in terms of the radiological effect, the brain was fairly insensitive. However, the chemical effect on the brain is uncertain and needs to be studied in more depth. Dr. Haley asked if there were estimates as to how much (percentage-wise) of DU went to the brain. Mr. Marshall indicated this needed to be investigated further, but he would hypothesize it to be in range similar to other soft tissue organs.

Mr. Robinson asked if Mr. Marshall thought, based on his findings, it would be a good idea to conduct a more in-depth study of the effects of DU on veterans, along with studies on the current conditions at locations involved in the first Gulf War. Mr. Marshall thought both studies needed to be conducted to put these lingering issues to rest.

Dr. Sushil Sharma, U.S. General Accounting Office (GAO), asked Mr. Marshall whether he had determined the inhalation particle size effect. Mr. Marshall stated that particle size did have an impact, but sensitivity studies still needed to be done to flesh out this concern.

Dr. Sharma asked about the extrapolation models used by Mr. Marshall. Mr. Marshall stated he used standard radiological settings, which were an extrapolation of much higher dosages. He indicated that there was considerable disagreement in the field as to what constitutes low-end exposure. Dr. Sharma asked whether Mr. Marshall had compared total DU intake vs. particle size. Mr. Marshall indicated that he had looked at these uncertainty factors.

Dr. Lewis enquired as to the solubility factors used in Mr. Marshall's calculations. Mr. Marshall stated that he hypothesized mostly U238, with some other isotopes, and had relied on tests that took the particulate amounts from various impact tests. He noted that, if one took a nominal case and predicted what amount of DU would be present in the urine seven to eight years later, he fell right on target with what was actually measured (on average). He stated that he was confident that his calculations were within a reasonable range.

An audience member inquired as to which DoD study provided the data for Mr. Marshall's calculations. Mr. Marshall stated it wasn't the "first" study, but the USACHPPM study. He stated that he had looked at all the impact data in order to make his calculations.

Chairman Binns thanked Mr. Marshall.

Overview of Armed Forces Radiobiology Research Institute Research on the effects of depleted uranium

Terry C. Pellmar, PhD
Scientific Director, Armed Forces Radiobiology Research Institute

Dr. Terry Pellmar presented an overview of the Armed Forces Radiobiology Research Institute's (AFRRI's) findings on the effects of depleted uranium. (See Appendix A – Presentation 11.) Dr. Pellmar noted that Dr. John Kalinich, her colleague, was in the audience.

During her discussion of Slide 11, Dr. Pellmar noted that, based on urine data comparisons, a four-pellet DU dosage in the rats was comparable to the highest level of Gulf War veterans' exposure. She also noted that a steady state in urine and kidney DU levels was reached after six months. However, the DU levels in bone continued to increase throughout the life of the animal. As the rats continued to grow and experience bone elongation, she wasn't sure how this would translate into adult humans.

With respect to kidney function, Dr. Pellmar stated that they found no evidence that DU adversely affected the kidney. She stated that this wasn't what they expected, and that a possible explanation might be enzyme up-regulation or perhaps the DU was being sequestered in some manner.

Dr. Liu and Dr. Melling questioned the animal (rat) model chosen without knowledge of the toxicological mechanisms. Dr. Pellmar indicated that, based on their findings' correlation with Dr. McDiarmid's Gulf War veteran findings, they were confident about their choice of model organism.

Following the talk, Dr. Pellier asked whether AFRRI had studied the effect of the pellet shape on DU uptake. He noted that pellets with higher surface area might result in higher exposures. Dr. Pellmar stated they hadn't performed this study, because of the difficulties in maintaining controls. However, she noted that, while the pellets were originally smooth, the evidence showed the pellets began to disintegrate quickly, increasing the overall surface area.

Dr. Haley questioned the focus on LD50 dose effects, rather than individual dose effects. He stated it would be interesting to look at the variable results in individual animals because there was indication that there could be increased individual susceptibility. Dr. Pellmar found this to be an interesting point, but noted it wasn't clear with these findings whether the variances were due to animal or measurement issues. She stated extra/multiple replicates were necessary to say definitely it was an animal variability issue. Dr. Haley referenced a previous study, involving marmosets and sarin that used this approach.

Dr. Haley asked how long did they (Dr. Pellmar's group) expect uranium to be excreted in humans. Dr. Pellmar stated that the studies hadn't been done, but she expected DU would be excreted until the pellet's reservoir was depleted.

Dr. Sharma (GAO) asked about the results of DU/sarin experiments. Dr. Pellmar stated that AFRRI hasn't worked with sarin.

Mr. Steve Smithson asked what happened to fragments in Gulf War veterans. Dr. Pellmar stated it was difficult to say, but she expected the pellets to be acting as a DU reservoir. Dr. Steele asked, if this was the case and based upon their mutagen and transformation findings, could there be reason to recommend removal of the DU fragments from the veterans. Dr. Pellmar stated, in her personal opinion, unless major problems with a particular fragment arose, the fragment should be left alone. Dr. Pellier questioned this position, because her data showed DU had mutagenic effects and they hadn't established a "no effect" level. Dr. Pellmar stated that the basis for her opinion was the lack of change in the rats' lifestyle and cancer rates. Colonel Jarrett, director of AFRII's laboratory and an Army emergency room surgeon, was in the audience and concurred with Dr. Pellmar's position. He stated that the current protocol was to take out easily accessible fragments, but leave in fragments when the removal process would cause extensive damage.

An audience member asked whether bacterial films had been observed surrounding the transformed cells. Dr. Pellmar stated that bacteria weren't observed, but fibrous tissue had been.

Mr. Robinson inquired whether AFRRI had conducted any tests in Iraq or other places where troops might have been exposed to DU. Colonel Jarrett stated that this type of study was not in AFRRI's mission. He noted that when they did venture into DU research, they were stepping somewhat outside their range. Dr. Pellmar also noted that AFRRI's mission was to do basic, not clinical, research.

Mr. Dan Fahey asked whether time frame matching had been considered between the rat model and Gulf War veteran data. Dr. Pellmar noted two things she felt addressed this concern: (1) the DU animal group's levels were equivalent to the highest-level seen in veterans; and (2) the animal model's DU excretion levels leveled off (became steady) after six months.

Chairman Binns thanked Dr. Pellmar.

The meeting adjourned at 10:00 a.m. for a break.

The meeting reconvened at 10:15 a.m.

Inhalation of uranium oxides to mimic Gulf War exposures: Deposition and toxicity in brain, lung, and kidney

Johnnye Lewis, PhD, DABT
Director, Community Environmental Health Program, University of New Mexico Health Sciences Center

Dr. Johnnye Lewis provided the Committee with background as to how her team was investigating the inhalation of uranium aerosols, and addressed their early findings. (See Appendix A – Presentation 12.)

Following the talk, Dr. Golomb asked if Dr. Lewis had considered a correlation between this research and reported symptoms of MCS patients exposed to pesticides. Dr. Golomb noted that affected female-to-

male ratios were high in MCS too. Dr. Lewis indicated that obtaining tissue samples would be relatively easy, but deciding how to test would be harder.

Dr. Haley enquired as to the high mortality rate in the study's female rats. Dr. Lewis stated that this was not an anticipated result, and they were not sure what the possible reasons were.

Dr. Haley asked if it was known how long the DU aerosol spray stays suspended in the air. Dr. Lewis indicated that this had been a consideration in their study. She stated that there was data in the literature on this, and noted that the time was dependent on the size of particles generated.

Mr. Graves asked whether the solvents, e.g., oil from fires, in the atmosphere might have increased a soldier's DU uptake. Dr. Lewis stated that they were beginning to characterize some of these particulate distributions.

Chairman Binns thanked Dr. Lewis.

Health effects of depleted uranium in exposed Gulf War veterans – A ten-year follow-up

Melissa McDiarmid, MD, MPH
Director, Depleted Uranium Program, Baltimore VA Medical Center

Dr. Melissa McDiarmid presented an overview of VA's DU surveillance program, along with data (including the most recently published 2001 data) collected from veterans involved in friendly fire incidents. (See Appendix A – Presentation 13.)

Towards the beginning of Dr. McDiarmid's talk, Mr. Anthony Principi, Secretary of the Department of Veterans Affairs, arrived. Dr. McDiarmid graciously yielded the floor to Secretary Principi. Secretary Principi apologized for the interruption, and stated he just wanted to stop by and thank Chairman Binns and the rest of the Committee for their hard work and effort to find conclusions to the health issues that Gulf War veterans have been grappling with for a long time. He indicated that he had been spending a lot of time with returning OIF soldiers at Water Reed and Bethesda hospitals. While many of these soldiers' injuries involve shrapnel, he stated that there are many environmental hazards on this war's battlefield, as there was in the first Gulf War. For this reason, he stated that the Committee's work was very important in identifying the issues, as well as the research to answer these concerns. He stated that there simply was no more urgent mission than to find answers for the men and women who commit themselves on the battlefield and to help devise ways to protect our troops. He stated that he was committed to this Committee's effort.

Dr. McDiarmid returned to her talk, explaining the surveillance protocol for her group's study. During her discussion of the 2001 cohort renal function findings, she also discussed the urinary markers for toxic nephropathies identified in the European Cooperative Study. Mr. Graves asked whether there was a correlation between the lead/heavy metal data and emerging DU data. Dr. McDiarmid stated that, in terms of prognosis and the hierarchy of heavy metal renal insults, uranium would be towards the bottom of the list. Mr. Graves asked if there was any form of treatment, e.g. detoxification. Dr. McDiarmid stated there wasn't.

Towards the end of her talk, Dr. McDiarmid provided an overview of the VA's nation-wide uranium sampling program. She described the process by which veterans mail in urine samples for testing. She presented data from 446 GWV (three of which were in the original cohort of friendly-fire veterans) and 147 OIF veterans (Air Force and Marines). Their preliminary findings indicated that these veterans'

creatinine levels were within appropriate ranges. Mr. Smithson asked if any of the OIF veterans retained shell fragments. Dr. McDiarmid indicated that this data was coming to them in pieces at this time, due to problems with centralizing communication between VA and the armed forces branches. She indicated that, based upon this data, she believed there wouldn't be a health risk problem associated with DU in the future.

Chairman Binns thanked Dr. McDiarmid. He apologized to the Committee and audience for the lack of flexibility in the meeting's time schedule for a formal discussion/question period. Dr. McDiarmid offered to stay and answer individual questions privately.

The meeting adjourned at 12:07 p.m. for lunch.

The meeting reconvened at 1:07 p.m.

Committee Business

Chairman Binns asked the Committee to take time at that point for discussion, specifically as to their reactions to the VA funding status report heard the previous morning. Chairman Binns stated that he had hoped to have this discussion before meeting with Secretary Principi. However, their meeting had been rescheduled, and he had just come from the Secretary's office. He stated that he presented some ideas expressed to him by Committee members the previous day.

The Committee discussed their concern about the lack of GWI-related studies funded by VA. They expressed concern that this research had been "disguised" under the deployment health initiative and had likely not interested field researchers, and had not been reviewed by peer-review panels that had special knowledge about GWI. Dr. Haley spoke about the Juvenile Diabetes and NIH AIDS vaccine research funding projects. He indicated that both groups had spent time reviewing the current research and tailored funding announcements to invite numerous, exciting proposals. Dr. Melling noted that a focus strategy was needed when developing funding announcements. Otherwise, the approach will diffuse the resulting pool of proposal applications.

Mr. Graves stated that, in his opinion, VA ORD hadn't spent any money on GWI, but believed there was still a chance this year for the Committee to get involved and change things. Dr. Golomb suggested the funding announcement list the types of research that were important for breakthroughs, e.g. treatment, acetylcholinesterase, etc., but also indicate a non-interest in proposals that focus on stress/psychological research. Dr. Melling suggested that, at the next Committee meeting, there be a short session to discuss what research models succeeded, and why they were able to accomplish what they did.

Chairman Binns pointed out that time was of the essence, both with the end of the fiscal year approaching and possible administration changes later in the year. He stressed that this was an urgent problem. He suggested that the Committee provide VA ORD with needed expertise in this area. He found urgency, earmarked funds, and a special Gulf War review panel to be the ingredients that would turn things around. He noted that VA had the Committee's initial recommendations, and needed to push forward. Discussion occurred about the need to encourage collaborations with outside (non-VA) researchers. The Committee discussed how this would help widen the research pool, as well as knowledge/ideas.

Chairman Binns stated he was pleased with this discussion. He stated that he was able to offer positive ideas to the Secretary, while getting across displeasure at the way the current DHI announcement was

handled. Chairman Binns stated that all of the parties want to address GWI concerns, and the key was working together to address these concerns.

Discussion occurred about the VA field researchers' disillusionment with submitting proposals in this area of research. Chairman Binns noted the dichotomy between the Committee hearing every meeting from scientists doing exciting work, but that when VA funding doors were opened, nobody came forward.

Overview of Research on Infectious Diseases in Gulf War veterans

Lea Steele, PhD
Scientific Director, Research Advisory Committee on Gulf War Veterans Illnesses

Dr. Steele provided a brief overview of GW-related infectious disease research being done. (See Appendix A – Presentation 14.) Most of this research has focused on leishmania and mycoplasma infections.

Antibiotic Treatment of Gulf War Veterans' illnesses

Sam Donta, MD
Retired Professor of Medicine, Boston University School of Medicine

Dr. Sam Donta, as principal investigator (PI) on the VA's antibiotic treatment trial on Gulf War veterans, provided an overview of the study's results, as well as his insights into the study's development, design and technical issues. (See Appendix A – Presentation 15.)

Dr. Donta noted that this study was initiated, in part, in response to Congressional concerns about Dr. Garth Nicolson's mycoplasma findings. Dr. Donta stated that he was asked to be on the study's committee, because he had been a PI on a couple of other infectious disease studies, and also because of his work in Lyme disease. He stated that he hoped the scientific community now was in agreement that the Gulf war veterans were really sick, by any parameter.

Dr. Donta provided a history of the development of the study's design. At first, the researchers thought they might combine the antibiotic and cognitive behavior therapy (CBT) trials. But ultimately, this was rejected. The results of the CBT trial (published in JAMA) showed initial improvement in the Gulf War veterans' health. Dr. Donta noted that there was concern about the basis for the initial study hypothesis but that subsequent studies had provided support for the hypothesis.

Dr. Donta stated that the focus on the cause of GWI might be too narrow. He noted that the focus had been that these veterans were in the Gulf War, and therefore they "got" something from the Gulf War. He acknowledged this is possible, but suggested that it also could be a reactivation of a previously latent process, one which is reactivated by stress. He noted that the fibromyalgia, chronic fatigue syndrome and Lyme disease literatures were filled with cases where people were asymptomatic, and after going through a stressful process, reactivated a latent disease. He stressed that there could be several causes behind GWI. He noted that chemical assaults should reach some stable level of damage. He noted, however, that an individual may have a chronic underlying infection, but see fluctuations in his or her symptoms.

Dr. Donta discussed the problems with specimen handling/processing. He also discussed why doxycycline was chosen as the study's antibiotic. He stated that his clinical experience indicated doxycycline might not work because it was highly protein bound. He had suggested tetracycline, while

various drug companies promoted the use of their respective antibiotics. He stated that doxycycline was finally selected, in part due to Dr. Nicolson's strong belief in its effectiveness.

Dr. Donta addressed the study's length of time, i.e. 12 months. He stated it was in part to deter criticism that the treatment hadn't been administered long enough. However, he noted that the period time also draw criticism that it was too long, in light of current antibiotics protocols.

During his discussion of the study's exclusion criteria (Slide 6), Dr. Donta stated that it is difficult to identify these types of intracellular infections. He indicated that there was a need for a tissue registry to improve understanding in this field.

During his discussion of the study's results (at Slide 13), Dr. Donta discussed criticisms raised about the study's control groups. He stated that they had considered using an additional control group of well, non-deployed Gulf War era veterans. However, he stated this had been rejected due to the concern of making the study too large. The study researchers' consensus was that they could control within the ill group appropriately (treatment vs. non-treatment). Dr. Donta noted that they had also considered providing treatment to PCR negative (-) mycoplasma patients. He stated that these were broad-spectrum antibiotics, and it might provide a clue to helping this patient group although he noted that duration of treatment would be a major concern.

Dr. Donta stated that adverse effects to the doxycycline were relatively few. He noted that nausea was more prevalent in the group receiving doxycycline, but, interestingly, myalgia symptoms were reported less than in the placebo group.

During his discussion of mycoplasma conversion rates (Slide 18), Dr. Donta acknowledged that the biggest conundrum was finding that a majority of both groups (doxycycline and placebo) had converted to mycoplasma negative (-) within eighteen months. He stated that additional study was needed to address quality control questions. He noted that the opportunity for PCR contamination was enormous. Discussion occurred about false negatives. Dr. Golomb noted that these results would suggest that less contamination occurred over time.

Dr. Donta noted that his group had looked for three mycoplasma subsets or species (*M. fermentans*, *M. genitalium*, *M. pneumoniae*). *M. pneumonia* proved to be the control species (no more than 5% of the ill Gulf War veterans evidenced this). *M. fermentans* and *M. genitalium* were present in more veterans, overwhelming so, which correlated with the study's hypothesis. Dr. Donta stated his belief that these test results were limited, due to the low levels detected. He did acknowledge, that repeat testing could be a problem too. As far as the mycoplasma subsets, he stated there wasn't enough data to show the variation between *M. fermentans* and *M. genitalium*. But the number of *M. pneumonia* didn't change.

Similarly, Dr. Donta stated there weren't enough data when they looked at doxycycline levels to provide statistical significance. He stated this needed to be fleshed out in further papers. When questioned if the patients were consistent in taking the doxycycline, Dr. Donta indicated he believed so. He noted that doxycycline had a long half-life, and if it was in the patient's system, they should have detected it even after several days.

Dr. Donta ended his presentation with two thoughts: (1) Even if a treatment study doesn't work, researchers still need to keep looking for the cause, including infectious agent causes. He stressed the need to look at low-level infection types, citing leishmania; and (2) the need to continue searching for treatment options. He suggested three/four month trials might be something to consider. This would provide a quick idea as to whether a treatment hypothesis has possibilities.

Following the presentation, Dr. Haley asked if serology was a reasonable marker of having been in contact with the organism. Dr. Donta stated that if one has an antibody to a particular organism, they would have been in contact. Dr. Golomb commented, though, that false negative (-) results with mycoplasma were common. Dr. Donta agreed; being sero-negative doesn't preclude previous contact.

Dr. Donta stated that mycoplasma, as a cause of GWI, could not be discounted totally by this study. Methodology issues needed to be addressed.

Chairman Binns asked if Dr. Donta, himself, had tried tetracycline treatment on ill Gulf War veterans. Dr. Donta indicated that he had, though not enough to constitute a study. Based on his observations, the veterans improved more on tetracycline than doxycycline, but the overall success rate still wasn't that good.

Dr. Donta stated that a pilot or smaller treatment studies needed to be done. He indicated his willingness to help design these studies. He suggested they be over a 20-week period with 20 patients in each group. He noted that the results might not help the first Gulf War veterans, but may help those returning from the current war. Dr. Donta also stressed the need to improve investigative tools to address concerns about processes, e.g., freezing/thawing of whole blood samples, might adversely affect the results.

Dr. Meggs asked if researchers needed to look at the idea of multi-stressors, i.e., if the person was "damaged" in some way, this might be compounded by their response to stress. Dr. Donta agreed that stresses do contribute to morbidity. Whether they help reactivate the genes was a question though. He indicated that he thought it was possible based upon his clinical experiences. He noted that biochemically it would be difficult to decipher.

Dr. Steele asked if Dr. Donta, in his clinical practice, had observed Gulf War veterans being more resistant to antibiotic treatment than Lyme disease patients. Dr. Donta stated the opposite was true; the Lyme patients who had been sick for more than 4 or 5 years were more resistant. He believed that duration of illness was the key. Dr. Steele asked if the reported improvements were sustained following treatment. Dr. Donta indicated yes. Dr. Steele also asked if there was a patient subset that improved substantially, which Dr. Donta affirmed. Dr. Golomb asked if some of the veterans, even those within the significant improvement group, experienced relapses later. Dr. Donta stated that some do. Dr. Golomb noted that this was compatible with the idea of another underlying factor. She stated that the infection added an additional detrimental function, but antibiotic treatment would only reverse the infection, not all factors.

Dr. Quentin Demming, who was in the audience, commented that, from his experience, it was possible that the study showed a successful suppression of a microbial disease.

Chairman Binns thanked Dr. Donta.

Review of recent (and recently identified) Gulf War research

Beatrice Golomb, MD, PhD
Asst. Professor, University of California at San Diego School of Medicine

Dr. Beatrice Golomb gave a brief review of recent Gulf War research, including discussion about the findings of the 2003 Australian Health Study and various other studies. (See Appendix A – Presentation 16.)

Committee Business

Dr. Steele commented that she was working on drafting the 2004 Committee report. She apologized for the lateness, indicating that it was larger than anticipated. She stated that she hoped to have a draft for circulation within the next month or so.

Dr. Steele also noted that the draft letter to the editors of *Military Medicine* was still pending. She reminded the Committee members to send her their comments, even if it was a just a confirmation of approval, as all Committee member names would be on the article.

Dr. Steele asked for the Committee's feedback on the format of the monthly update from Committee staff. The consensus was that they liked it. Dr. Steele noted that committee members were welcome to send news and research articles for inclusion in the update. She stated that the attachment size may be a problem for some members, and offered to send only abstracts if a member preferred. She mentioned that staff was working on website options. She also noted that the Committee's growing research library was probably one of the best anywhere. She stated that if a Committee member needed an article, they should feel free to contact staff.

Dr. Steele noted that the Committee had a new e-mail address for public inquiries and submissions: RAC@med.va.gov.

Chairman Binns asked whether the Committee's updates could be made available to the public. Dr. Steele and Ms. Palmer indicated that they were investigating this possibility.

An audience member asked about the availability of the presenters' slides. Dr. Steele stated that the slides would be available in the minutes. She noted that some might be slightly modified by the presenter if a slide contained unpublished data.

Chairman Binns commented that the meeting had been of high caliber. He noted that Dr. Steele already was working on the upcoming meeting's schedule. He stated that with all the knowledge out there, he believed that things would gradually start to piece together. However, he also welcomed Dr. Donta's comment that treatments shouldn't wait until the whole puzzle has been deciphered.

Public comment – Day 2

Ms. Venus-valiery Hammack, Army Retired, stated that she had prepared her comments in writing. However, she indicated that she needed to amend them and would provide them at a later date. She noted that the Committee had covered her issues about the implementation and follow-up on the Committee's previous recommendations to VA.

Dr. Steele noted that the Committee was inviting people to supplement their oral testimony with written two-page (or less) statements/summaries.

Next, Ms. Denise Nichols addressed the Committee with her concerns about GWI research. She also provided the Committee with a two-page written comment. (See Appendix B – Presentation 2.) She stated that administrators/researchers needed to do a computer match-up, by Social Security number, to have a more accurate number of deaths. She stated her belief that there were more Gulf War veteran deaths than those being counted. Dr. Steele stated that the GWVIS report did report only total numbers,

but the on-going epidemiological studies did do matches using Social Security numbers. Ms. Nichols thanked Dr. Steele for the clarification.

Ms. Nichols informed the Committee about the recent death of a Gulf War veteran, who was a part of Dr. Haley's neurological study group. She suggested establishment of a mortality review committee, similar to those found in hospitals. She stated that this type of review might provide missing clues to why Gulf War veterans are sick. She suggested more research into the triglyceride/cholesterol levels of Gulf War veterans. She also stated that many have very high levels, and several are dying of sudden heart attacks. She asked whether this might be due to an early aging factor. She stressed the need to investigate new paths in this area of research.

Ms. Nichols stated that the quality of VA care for Gulf War veterans was very low. She stated the lack of care created frustrations for the ill veterans. She suggested making a panel to review the quality of care for Gulf War veterans. She stated that she was working with veterans to gain better access to healthcare and VA benefits. She stated that their military administration folders were missing important documents, causing the veterans many problems, including denied claims.

Ms. Nichols suggested that more research be done on Gulf War veterans' magnesium levels. She noted similar problems in CFS patients and individuals who had undergone radiation therapy. She suggested going back and looking at old radiation studies for clues.

Ms. Nichols noted her appreciation for the Committee's efforts. She stated that the Committee was working hard and trying to make process, but outside influences hadn't helped it along. She stated that she had enjoyed the meeting's presentations.

Chairman Binns thanked Dr. Steele for coordinating the meeting presentations. He then asked if there were any final comments or questions.

Mr. Robinson stated that he, along with some others, had a conversation with Dr. McDiarmid following her presentation. He stated that they had wished to discuss some outstanding issues regarding the VA's DU program. He indicated that she had not been open to discussing some of their questions. He asked if the Committee could send her a list of follow-up questions and request written responses. Chairman Binns indicated that this was possible. He stated that Committee members could send questions to the Committee's staff and then circulate the final list before submitting it to Dr. McDiarmid. Dr. Steele also indicated that Dr. McDiarmid could be invited back to a later meeting.

Chairman Binns thanked the Committee for its hard work, and noted the members' outstanding attendance record.

The meeting adjourned at 3:27 p.m.

Appendix A
Presentation 1 - John Concato

ACETYLCHOLINESTERASE ACTIVITY IN GULF WAR DEPLOYED AND ERA VETERANS: FEB '04 UPDATE

John Concato, M.D., M.S., M.P.H. (West Haven)
Bradley Doebbeling, M.D., M.Sc. (Indianapolis)
Peter Peduzzi, Ph.D. (West Haven)
Hermona Soreq, Ph.D. (Jerusalem)
Catherine Viscoli, Ph.D. (West Haven)

BACKGROUND RE: SOURCES OF DATA

Questionnaire responses from Iowa Gulf War Cohort Study
- Wave I: 3,695 veterans of Persian Gulf era, from Iowa
- Wave II: 374 case patients with cognitive dysfunction, depression, or chronic widespread pain; 228 controls without these conditions (N=602 subset of Wave I)

Laboratory analyses of stored sera, at Hebrew University
- Acetylcholinesterase (AChE)
- Butyrylcholinesterase (BChE)
- Paraoxonase, Arylesterase (PON1, Aryl)

RESEARCH HYPOTHESES

1. Mood and anxiety symptoms are associated with selected blood enzyme levels

2. Deployed (vs. non-deployed) Gulf War veterans have lower capacity to increase blood AChE levels

3. Veterans with (vs. without) symptoms of Gulf War Veterans Illness (GWVI) have lower capacity to increase blood AChE levels under challenge

OVERVIEW OF CURRENT STATUS

- Transfer of questionnaire data, review of data dictionaries, definition and coding of variables completed

- Blood samples shipped; laboratory analyses of AChE, BChE, PON1, and Aryl enzymes completed

- Analyses of initial hypotheses conducted; other analyses ongoing or under consideration

FINAL STUDY SAMPLE FOR CURRENT ANALYSES

Wave II participants:		602
• enzymes not measured	- 25	
• non-white or female veterans	- 89	
		-114
Final study population:		488

FORMAT FOR CURRENT ANALYSES

- Enzyme levels assigned as outcome variables in multiple linear regression analyses; results presented as predicted least square mean values (nmol/min/ml) for each enzyme

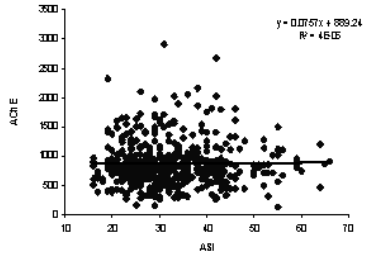
- Models done both unadjusted and adjusted for:
  a) age, BMI, smoking, acute illness
  b) *plus* antidepressant medications or alcohol/drug use
  c) *plus* case-control status in original Iowa study, for analyses of deployed vs. non-deployed veterans

1. ASSOCIATION OF ANXIETY AND ENZYME LEVELS

Stress responses involve several neural pathways, and disease-associated changes in AChE and other enzymes might be expected among patients manifesting anxiety or mood symptoms

Prior work (in a "healthy" population) found a strong negative correlation between anxiety measures and AChE

ASSOCIATION OF ANXIETY AND ENZYME LEVELS



CURRENT ('STATE') AND LIFETIME ('TRAIT") ANXIETY

Predicted, adjusted mean enzyme levels (and P values), N=476:

	AChE	BChE	PON1	Aryl
Current:				
Yes	887	5038	45.7	17.0
No	893	5155	41.8	17.6
	(0.88)	(0.48)	(0.16)	(0.49)
Lifetime:				
Yes	884	5042	45.1	17.1
No	895	5162	41.7	17.6
	(0.80)	(0.45)	(0.19)	(0.55)

2. ASSOCIATION OF DEPLOYMENT AND ENZYME LEVELS

Prior work suggested that external stimuli (e.g., extreme weather, stress, medications) could induce activation of AChE expression and affect enzyme activity

Deployment to the Persian Gulf region could be associated with a decrease in AChE enzyme activity due to enhanced degradation or suppressed production

ASSOCIATION OF DEPLOYMENT AND ENZYME LEVELS

Predicted, adjusted mean enzyme levels (and P values), N=476:

	AChE	BChE	PON1	Aryl
Deployed				
Yes:	885	5069	42.5	17.2
No:	913	5304	43.3	18.3
	(0.49)	(0.14)	(0.74)	(0.16)

3. ASSOCIATION OF GWVI AND ENZYME LEVELS

Pertinent symptoms reported at Wave I or II were linked to CDC criteria for GWVI (e.g., involving 2 of 3 axes re: mood-cognitive, fatigue, musculoskeletal symptoms)

GWVI: Definition 1 - onset of symptoms after Gulf War, present at Wave II, regardless of status at Wave I; Definition 2 - onset after Gulf War, present at Wave I and Wave II (subset with longer duration of symptoms)

Analyses of GWVI done for deployed and non-deployed veterans

GULF WAR VETERANS ILLNESS: DEFINITION 1

Predicted, adjusted mean enzyme levels (and P values), N=476:

	AChE	BChE	PON1	Aryl
Deployed				
GWVI	892	5015	42.8	17.4
No GWVI	878	5165	42.2	17.0
	(0.78)	(0.41)	(0.85)	(0.67)
Non-Dpld				
GWVI	890	5107	43.8	18.1
No GWVI	928	5595	41.7	18.4
	(0.61)	(0.14)	(0.72)	(0.85)

GULF WAR VETERANS ILLNESS: DEFINITION 2

Predicted, adjusted mean enzyme levels (and P values), N=474:

	AChE	BChE	PON1	Aryl
Deployed				
GWVI	933	4979	41.4	17.1
No GWVI	854	5129	43.5	17.4
	(0.08)	(0.38)	(0.46)	(0.70)
Non-Dpld				
GWVI	965	5207	35.6	16.5
No GWVI	886	5341	45.1	18.7
	(0.32)	(0.70)	(0.11)	(0.24)

SUMMARY

Findings not consistent with original hypotheses regarding association of anxiety or mood disorders and AChE or other enzymes in this population

Deployment status did not affect AChE or other enzymes

Symptoms of GWVI were not strongly associated with AChE or other enzymes

Presentation 2 - Roger Kaplan

VA Gulf War Illness Research Project

Roger Kaplan, Special Assistant
Office of Research and Development

- "Effects of Gulf War Illnesses on Brain Structure and Function" Mike Weiner, MD, San Francisco VAMC

- FY 02-07 ($3 Million)

- Neuroimaging study will examine structural changes in basal ganglia and pons of healthy and ill Gulf War Vets

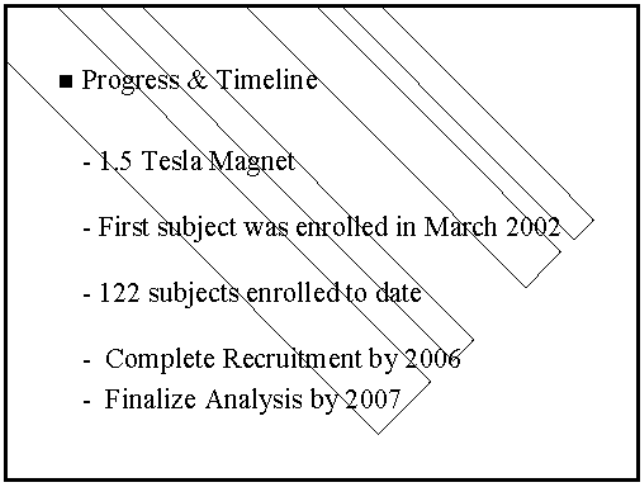
- Replicate findings in a previous study by considering PTSD, alcohol abuse, and depression as covariables

HYPOTHESES

- **Hypothesis 1:** *Subjects with GWI have abnormalities independent of the effects of the confounds of alcohol abuse, PTSD and depression.*
- ***Hypothesis 2:*** *NAA reductions in the basal ganglia and pons correlate with measures of cognition, audio-vestibular function, enzymes involved in organophosphate metabolism, and dopamine metabolism.*

EXAMINATIONS

- Physical and Neurological
- Neuro-psychological
- Haley GWI Questionaire
- PTSD Assessment
- Audio-Vestibular (ECoG; ABR; Vestibular; ENG; SHA)
- MRI and MRSI


■ Progress & Timeline
- 1.5 Tesla Magnet
- First subject was enrolled in March 2002
- 122 subjects enrolled to date
- Complete Recruitment by 2006
- Finalize Analysis by 2007

Presentation 3 - Preeti Hans

VA's Deployment Health Initiative

Preeti Hans, MHP
Gulf War Illnesses
VA Central Office
Office of Research & Development

Description of Research Projects Funded under the Deployment Health Initiative in Late FY 03 and FY 04

- Biomedical Laboratory Research and Development (BLR&D)
- Clinical Science Research and Development (CSR&D)
- Rehabilitation Research and Development (RR&D)

- Health Services Research and Development (HSR&D)
- Cooperative Studies Program (CSP)

- Fall 2002 Deployment Health Research Solicitation was Announced by BLR&D and CSR&D
  - One project titled "The Role of Dietary Choline in Neuroprotection" was funded for three years beginning in FY 04 ($450,000)
  - Three projects did not receive funding from the 2003 Fall Review
  - Cut off scores for the Services was a score below 22

RR&D

- RR&D Scientific Review occurred in Jan 04
  - Two proposals have received conditional funding
  - RR&D usually has a funding cut-off score of 21

HSR&D

- HSR&D Review occurred in Jan 04
- 2 Proposals were not funded
- HSR&D usually has a funding cut-off score of 22-20

HSR&D

- "Neuropsychological Assessment of a Population-Based Sample of Persian Gulf War Veterans and Controls" Mitchell T. Wallin, MD, MPH
  - Goal of study is to understand the neurocognitive health of Gulf War veterans
  - FY 02-04 ($100,000)

CSP

- Solicitation for Persian Gulf War Veterans' Illness Research was announced in Fall of 1997 and then re-announced in Winter of 2000
- Hypothesis-driven, multi-site, randomized clinical trials to evaluate treatments proposed for Veterans with GWI
- 5 Major Studies concerning ALS, EBT, ABT, Millennium Cohort Study, and a National Health Survey of Gulf War Era Veterans & their Families have been funded

CSP

- EBT Study funded for $9.2 Million Dollars from 1999-2001
  - 1100 veterans with GWI for assessment on the independent impact of aerobic exercise and cognitive therapy, on relieving symptoms of GWI (pain, fatigue, cognitive difficulties)
  - 2x2 Factorial design
  - Both treatments, especially exercise resulted in improvement in fatigue, cognitive symptoms, distress and mental health functioning (JAMA March 2003)

CSP

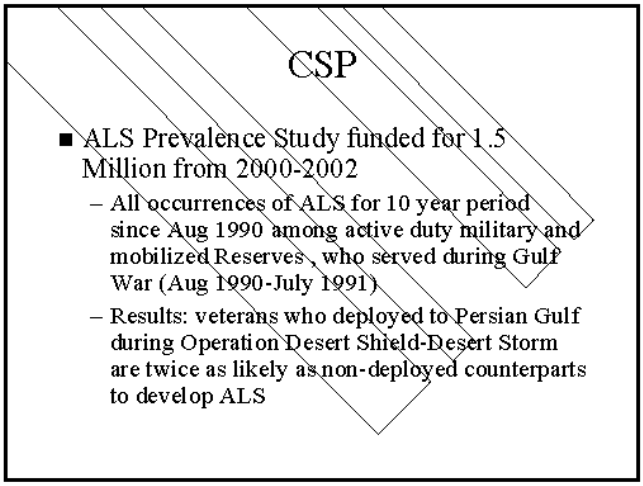
- ABT Study funded for $7.8 Million from 1999-2002
  - 500 Veterans with GWI who tested positive for mycoplasma
  - Determine effectiveness of doxycycline on reducing symptoms of GWI and lowering blood levels of mycoplasma
  - Results: No significant difference between treatment with doxycycline and treatment with placebo

CSP

- National Health Survey of GW Era Veterans and Their Families was funded for $11.9 Million (1998-2001)
  - Examination of physical health of 1,000 deployed GW veterans and their families compared with 1,000 non-deployed veterans and their families
  - Results are embargoed pending publication

CSP

- Millennium Cohort Study (2.1 Million from 2001-2002
  - Cross-sectional sample of 100,000 US military personnel (Oct 2000)
  - Prospective study --survey every 3 years
  - Objective: compare change in health status between deployed and non-deployed personnel and incidence rates of chronic disease between cohorts


CSP
ALS Prevalence Study funded for 1.5 Million from 2000-2002
All occurrences of ALS for 10 year period since Aug 1990 among active duty military and mobilized Reserves , who served during Gulf War (Aug 1990-July 1991)
Results: veterans who deployed to Persian Gulf during Operation Desert Shield-Desert Storm are twice as likely as non-deployed counterparts to develop ALS

Presentation 4 - Joe Gough

Fall 2002 Deployment Health Solicitation and Review Process

Joe Gough, M.A.
Acting Director of Administration
VA Central Office
Office of Research & Development

Solicitation Objective

- Expand ORD's systematic paradigm linking basic research to practice:
  - Epidemiology
  - Diagnosis
  - Treatment
  - Prevention of deployment-related illnesses

Categories of Research Focus

- "Long-term" effects of exposure/risk factors among deployed veterans
- Impacts of specific occupational and environmental exposures
- Advancements in evaluation, diagnosis, and treatment of deployment-related illnesses
- Health risk "communication" for veterans and health care providers

Biomedical Laboratory Science Research & Development

- Pilot projects of existing problems with new lines of investigation
- Differ from traditional proposals (preliminary data optional if rational hypothesis, literature, or logical basis)

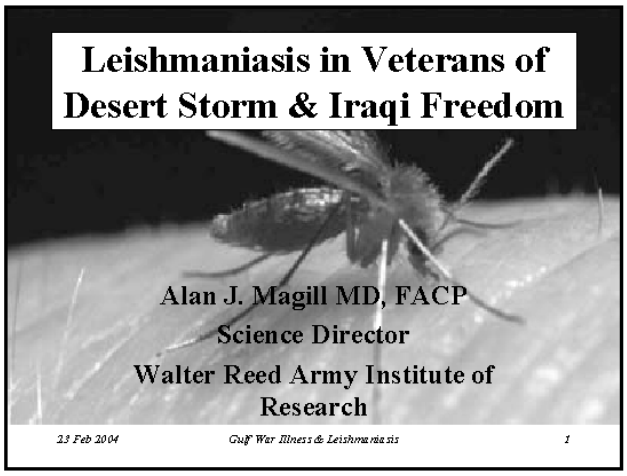
Peer Review Evaluation

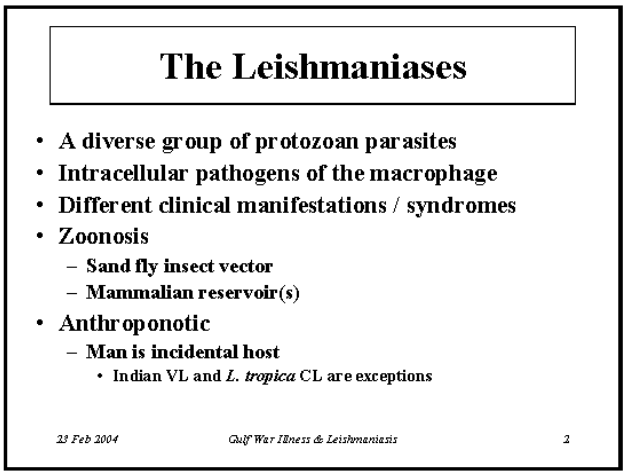
- Scientific quality
- Significance/Innovation
- Methodological rigor
- Feasibility
- Relevance to program announcement

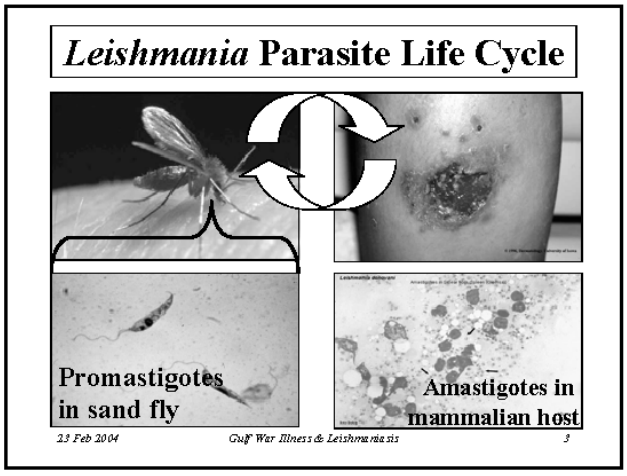
Common Peer Review Process

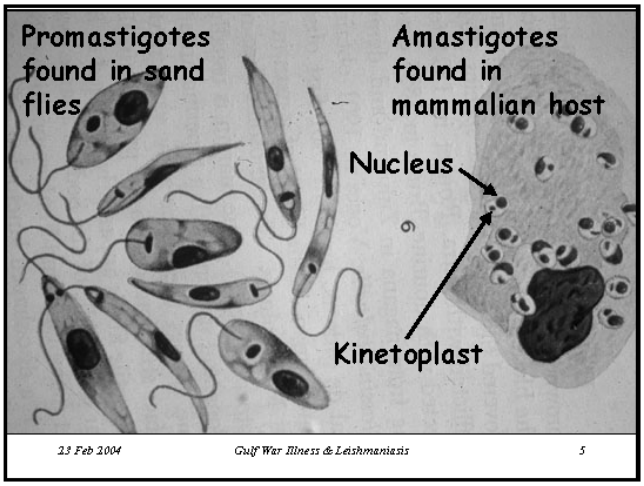
- OMB/OSTP – fund "best science"
- Service-specific boards ~ 15 members
  - scientific content "matched" to reviewer expertise
  - 3 reviewers/study
  - scoring - 10-50 scale (typically)
  - CRADO reviews aggregate scores

Presentation 5 - Alan Magill









23 Feb 2004 Gulf War Illness & Leishmaniasis 5

Leishmania infection and 1990-91 Gulf War

- What did we expect to see?
  - NEJM article, 21 Mar 1991. 324; 859
- Typical Cutaneous Leishmaniasis
  - *L. major* parasites
  - Desert rodent reservoir
  - *Ph. papatasi* sand fly vector
- N = 20 cases
- Visceral Leishmaniasis not described

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Leishmania infection and 1990-91 Gulf War

- What else did we see?
- Atypical "viscerotropic leishmaniasis"
  - *L. tropica* parasites
  - Desert rodent or human reservoir??
  - Sand fly vector?
- N = 12 cases, parasitologically confirmed
- N = ?? cases total

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What was unusual?

- Did not expect to see VL in Saudi Arabia
- Atypical, non-specific clinical syndrome
  - Not typical Visceral Leishmaniasis
  - Smear negative, culture positive
- Isolation of *Leishmania* from bone marrow
- Characterization of isolates as *L. tropica*
- Difficult diagnosis, insensitive tests

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NEJM. May 13, 1993

Table 1. Clinical Presentation of Eight Male Patients with Visceral Leishmaniasis, at the Time of Confirmatory Culture.

Patient No.	Incubation Period (Mo)	Signs and Symptoms at Presentation	Fever	Abdominal Pain*	Malaise*	Fatigue*	Physical Examination
1	2	Adenopathy	Yes	++	+	++	Hepatomegaly, splenomegaly, adenopathy
2	1–4	Fever	Yes	+	++	+	Normal findings
3	2–8	Gastroenteritis	No	+++	+++	+	Splenomegaly
4	2–6	None	No	No	No	No	Normal findings
5	4–12	Chronic fatigue with hepato-splenomegaly	Yes	+	+	+++	Hepatomegaly, splenomegaly
6	7–14	Chronic fatigue with adenopathy	No	+	+	+++	Hepatomegaly, adenopathy
7	1–6	Mononucleosis	Yes	+/−	+++	+	Normal findings
8	3–12	Fever of unknown origin	Yes	+	++	++	Hepatomegaly, splenomegaly

*One plus sign indicates that the patient reported the symptom when questioned by the examiner; two plus signs, that the patient himself reported the symptom without questioning; and three plus signs, that the symptom was the primary one. Patient 7, represented by the plus–minus sign, reported abdominal pain of brief duration associated with diarrhea.

Leishmania in 1st Gulf War

- **Characterizations of *L. tropica* based on CAE of 21 enzymes**
- **3 clusters of *L. tropica***
- ***Am J Trop Med Hyg. 1993. 49:357***

Visceral Leishmaniasis

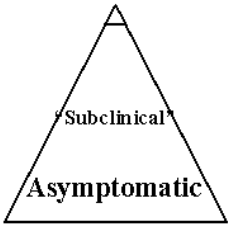
- ***Classic "pentad"***
  - **Fever**
  - **Cachexia**
  - **Splenomegaly**
  - **Pancytopenia**
  - **Hypergamma-globulinemia**
- ***L. donovani, L. infantum/chagasi***

Clinical Presentation of VL in the Immunocompetent,Well Nourished Adult

- ***Most and Levietes. 1947. Medicine. 26:221***
  - **N=30, US military in WWII**
  - **Incubation period: 3 weeks to 33 months**
  - **Symptom onset to definitive diagnosis, mean 10 weeks (range 2 -26 weeks)**
  - **Abrupt onset of fever and chills - 96%**
  - **Splenomegaly in 90% and hepatomegaly in 73% when Dx confirmed**
  - **Parasitologic diagnosis**
    - **21 of 49 (43%) smears from bone marrow aspirations in 29 patients were (+)**
    - **8 0f 29 (28%) were not confirmed by bone marrow**
    - **18 of 18 (100%) splenic aspirates were both smear and culture positive**

Visceral Leishmaniasis Disease Spectrum

1-3% with overt VL



- "Subclinical" Syndromes
  - Chronic systemic illness
  - Acute febrile illness
- Risk factors for progression
  - Malnutrition
  - Immunosuppression (AIDS)
  - Genetic?
- Cause of death
  - Measles
  - Pneumonia
  - TB
  - dysentery

"Subclinical Disease": Brazil

- Prospective pediatric cohort study, Bahia
- N = 86 seroconversion, 5 year follow-up
- 28 of 86 (33%) progressed to VL between 2 weeks and 15 months
- 20 of 86 (23%) remained asymptomatic
- 38 of 86 (44%) had a prolonged "subclinical" illness, resolved 35 mo on average
  - Intermittent hepatomegaly, diarrhea, failure to thrive, fatigue, malaise

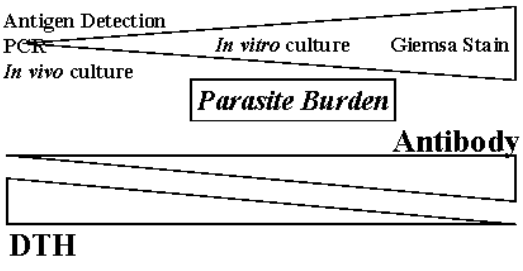
Badaro R, Jones TC, Lorenco R, et al; A prospective study of visceral leishmaniasis in an endemic area of Brazil. J Infect Dis 154;639, 1986

"Subclinical Disease"

- Perception of ill health and disease is culturally and resource dependent
- "subclinical" in the favelas of Brazil or rural Bihar, India = overt disease in suburban North America
- Cannot extrapolate reported experience in endemic areas to non-immune, immunocompetent adults

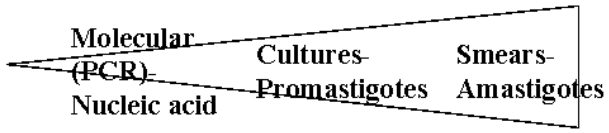
Spectrum of Disease : Diagnosis

Oligoparasitic *Polyparasitic*



Choice of Diagnostic Test?

- **Sensitivity of diagnostic test depends on parasite burden and thus clinical syndrome**



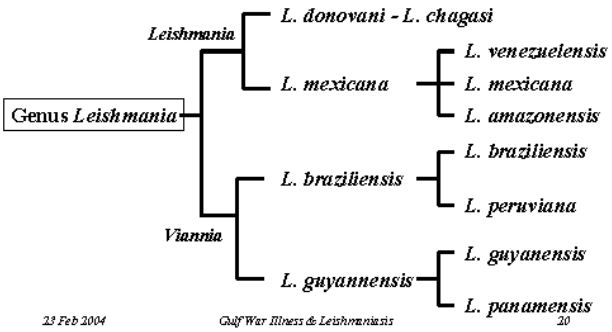
Other Systemic Syndromes: "Viscerotropic"

- Acute febrile illness
  - **Self limited?**
  - **Progressive to VL**
    - Weeks to months
- Adenopathy
  - **Localized, generalized, transient**
- Chronic gastrointestinal syndromes
- Failure to thrive

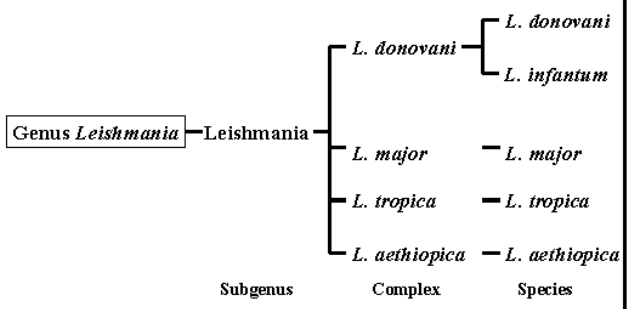
Diagnosis

- **Parasitologic diagnosis = confirmed diagnosis**
  - Smear, Culture, Antigen detection, PCR
- **Availability**
  - Routine clinical versus specialty labs
- **Clinical recognition**
  - Classic syndromes versus Gulf War *L. tropica*
- **Walter Reed Army Medical Center referral bias**

Taxonomic Classification of the Leishmaniases: New World



Taxonomic Classification of the Leishmaniases: Old World



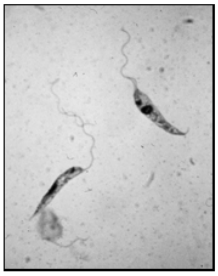
What is a Leishmania species?

- Classic definition
- Type organism isolated from a location and clinical syndrome (reference strain)
- Characterized by zymodeme analysis

L. tropica Reference Strains

	Where isolated	Source	International Ref No.	Clinical Manifestation
L. tropica	USSR Turkestan	Man	MHOM/SU/60/LRC-L39	CL
L. tropica	Iraq	Man	MHOM/IQ/00/Avraham	LR
L. tropica	Iraq	Man	MHOM/IQ/66/L75	CL

What is a zymodeme?

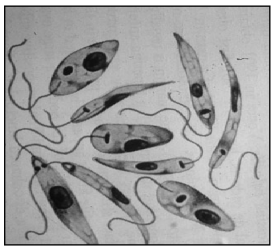


- Electrophoresis of parasite pellet
- 3 vs. 7 vs. 11 vs. 21 isoenzymes
- How different is different enough?
- Importance of minor enzyme allomorphs?

Other ways of classification

- PCR based methods
  - kDNA
  - rRNA
  - Repetitive nuclear sequences

They may look the same…



- Different parasites
- Different diseases
- Different epidemiology
- Different vectors
- Different reservoirs

Examples of Lt causing VL

- Kenya
  - Am J Trop Med Hyg 41:289
- Israel
  - Personal communication
- India
  - Ann Trop Med Parasitol 75:131
  - Lancet. 1995 Apr 15;345(8955):959-61
- Morocco
  - Ann Trop Med Parasitol. 2002 Sep;96(6):637-8.

L. tropica Genetic Heterogeneity

- Isoenzyme profiles of 27 stocks of Leishmania tropica from widely separated geographical areas were compared with those of reference strains of L. tropica and L. major using starch-gel electrophoresis of 13 enzymes (GPI, GD, ES, PGM, PEPD, NH, ASAT, ALAT, PK, MPI, 6PGD, SOD, MDH).
- 18 zymodemes were seen.
- L. tropica showed considerable intraspecific variation which did not correlate with its epidemiological uniformity.
- Isolates from cases of cutaneous and visceral leishmaniasis and leishmaniasis recidivans were identified as L. tropica.
- Only one isoenzyme band was held in common with the enzyme profile of the L. major reference strain thus supporting the status of L. tropica as a separate species.
- *Trans R Soc Trop Med Hyg. 1986;80(1):113-9*

Clinical heterogeneity: the Host

- **Near identical *L. tropica* isolates**
  - Nested PCR of kDNA
  - Restriction digests of amplicons
  - Shared fingerprint = schizodeme
- **Epidemic outbreak in a refugee camp**
- **21 isolates**
  - Nodular, 4-21 lesions, 1-12 mo duration
  - Outcome dependent on host response
- **J Clin Microbiol. 1998. 36:2877**

Can Cytokines Cause Disease?

- Acute disease
  - **Fever, malaise, myalgias, arthralgias, fatigue, anorexia, nausea**
  - **Influenza, dengue, malaria, tuberculosis, etc.**
  - **TNFα, INFγ, IL-2, IL-12, etc.**

Can Cytokines Cause Disease?

- Chronic disease
  - **Fever, malaise, myalgias, arthralgias, fatigue, anorexia, nausea**
  - **Inflammatory bowel disease, rheumatoid arthritis,**
  - **TNFα, INFγ, IL-2, IL-12, etc.**

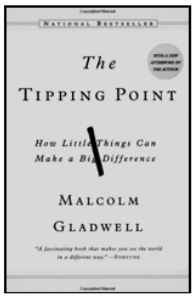
Side Effects of IFNγ as Therapy

- Constitutional: flu-like illness, fever, rigors, arthralgia, myalgia, fatigue
- Neuropsychiatric
  - **depression**
  - **insomnia**
  - **irritability**

Persistent *Leishmania* Infection

- Intracellular pathogen of the macrophage
- Lifelong, persistent infection
- Treat disease, never eradicate parasites
- Mycobacteria: TB, leprosy
- Bacteria: *Brucella*
- Fungal: *Histoplasma*
- Viral: *HIV*

The Tipping Point Malcolm Gladwell



- "Ideas and products and messages and behaviors spread just like viruses do"
- "... small numbers of people start behaving differently, that behavior can ripple outward until a critical mass or "tipping point" is reached..."
- Role of media

Presentation 6 - Ya Fang Liu

Assessment of a role of stress-activated kinases in the pathogenesis of Gulf War Syndrome

Ya Fang Liu M.D. & Ph.D.
Department of Pharmacology
Boston University School of Medicine

Part I

Introduction

Stress-activated kinases

They are a group of enzymes or kinases that are activated in response to stressful stimuli such as UV light, γ–irradiation, inflammatory cytokines, certain chemicals, toxins.

Activation of these kinases indicates that cells or neurons are undergoing cellular stress.

MKK4

- Mitogen-activated protein kinase kinase 4 (MKK4) controls activation of c-Jun-N-terminal kinase (JNK).

Physiological role of JNK

- JNK1 controls differentiation and cytokine production in Th2 lymphocytes (immune cells).
- JNK2 controls differentiation and cytokine production in Th1 lymphocytes.
- JNK1 and JNK2 are involved in the regulation of inflammatory responses

Physiological role of p38MAPK

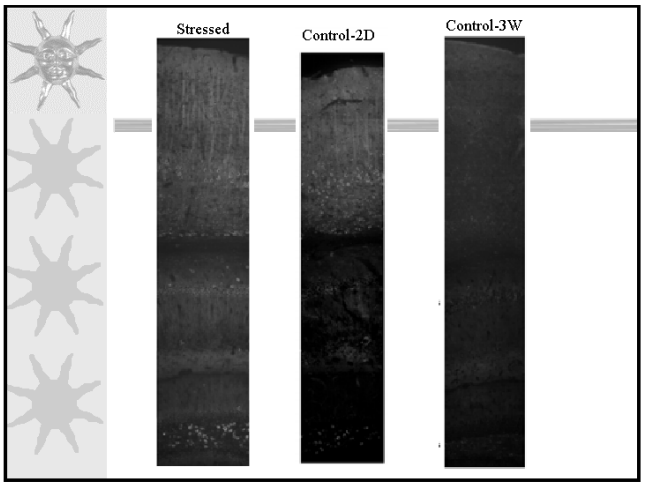
- Regulate differentiation and cytokine production in Th1 lymphocytes.
- Regulate inflammatory responses.

Pathological role of stress-activated kinases

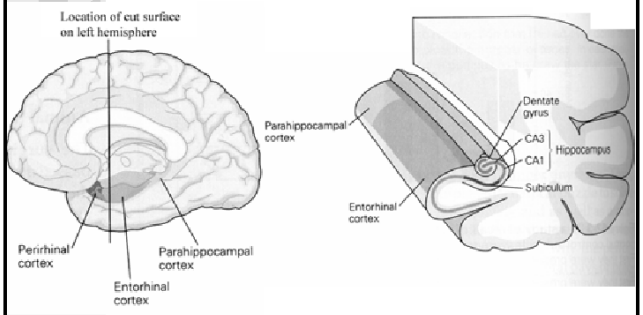
Over-activation of stress-activated kinases can induce dysfunction of central nervous and immune systems.

Stress

- Psychological stress;
- Physical stress;
- Environmental stress: cold, hot, new environment, transportation, high mountain attitude, high humidity, strange odors from chemicals or animals, noise, etc.
- Stress is a physiological response.
- Excess stress is the foundation of many illnesses.



Cognition and the hippocampal formation



Stress and the brain

- The Hypothalamus (Corticotropin-releasing factor,CRF)-Pituitary (adrenocorticotropic hormone,ACTH)-adrenal gland (cortisol): the HPA axis: stress responses.
- Dysfunction of the HPA axis has been implicated in chronic fatigue and sleeping disorders
- Amygdala: stress and emotional memory: dysfunction of the amygdala has been implicated in post-traumatic disorder (PTSD)

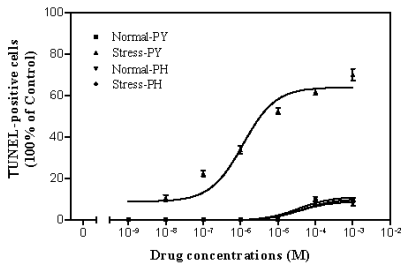
Part II

Hypothesis

Factors that may contribute to GWS

- Chemicals: such as pyridostigmine (PB), permethrin, DEET, serine, etc.
- Stress: physical stress, psychological stress, and environmental stress.
- Vaccinations: Challenge of the immune system.

Pyridostigmine is a potential neurotoxin



Pyridostigmine is a potential neurotoxin

- PB induces neuronal death when neurons are undergoing cellular stress or stress-activated kinases are activated. .
- PB-induced neuronal toxicity is independent of its inhibition of acetylcholine esterase.

Hypothesis

Stress, vaccination, and exposure to one or more chemicals may synergistically act on stress-activated kinases. Over-activation of these stress-activated kinases may lead to dysfunction in the central nervous and immune systems, contributing the majority of symptoms observed in patients with GWS

The Goal of the Project

- Identifying the molecular mechanism(s) underlying the pathogenesis of Gulf War Syndrome (GWS).
- Development of effective prevention and treatment for GWS.

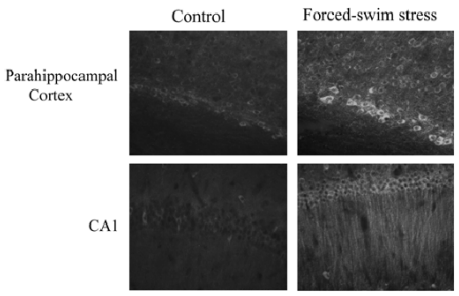
Part III

Stress Studies

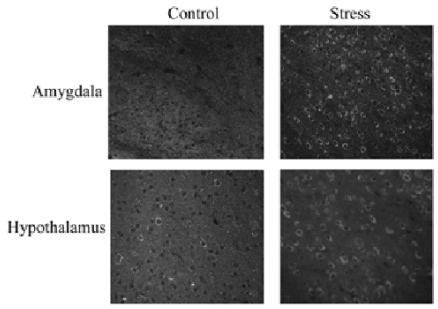
Forced-swim stress

- Physical stress
- Psychological stress
- Environmental stress

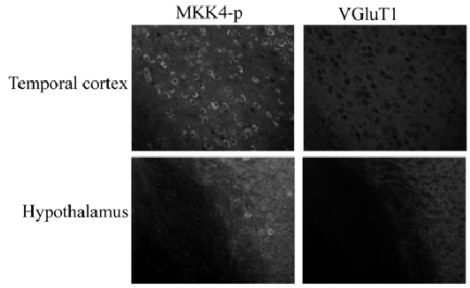
Forced-swim stress induces MKK4 activation in the cortex and the hippocampus



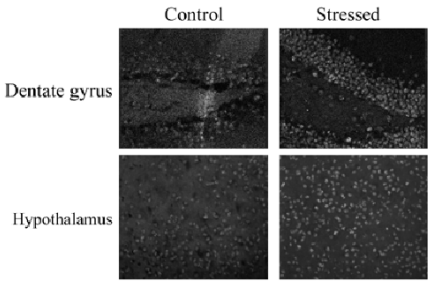
Forced-swim stress induces MKK4 activation in the hypothalamus and the amygdala



Forced-swim stress induces MKK4 activation in VGluT1-positive neurons



Forced-swim stress induces activation of JNK in the dentate gyrus and hypothalamus



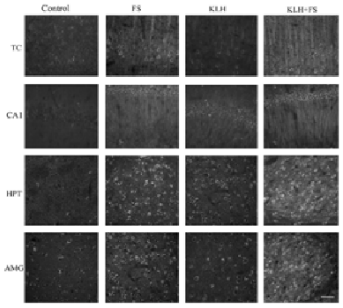
Summary

- Forced-swim stress induces activation of MKK4 and JNK in the hippocampal formation, the amygdala, and the hypothalamus.
- Forced-swim stress induces activation of MKK4 and JNK in glutamatergic neurons.

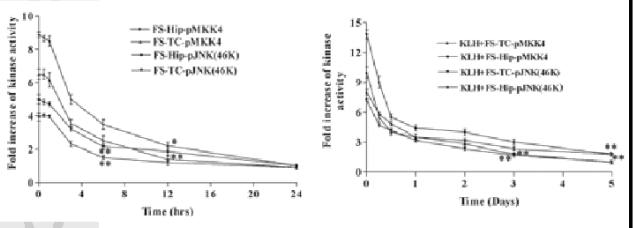
Part IV

Vaccination

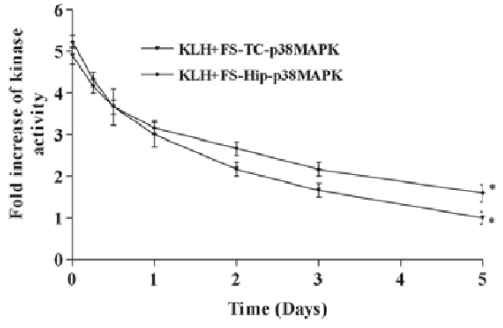
KLH immunization significantly increases activation of MKK4 in the brain

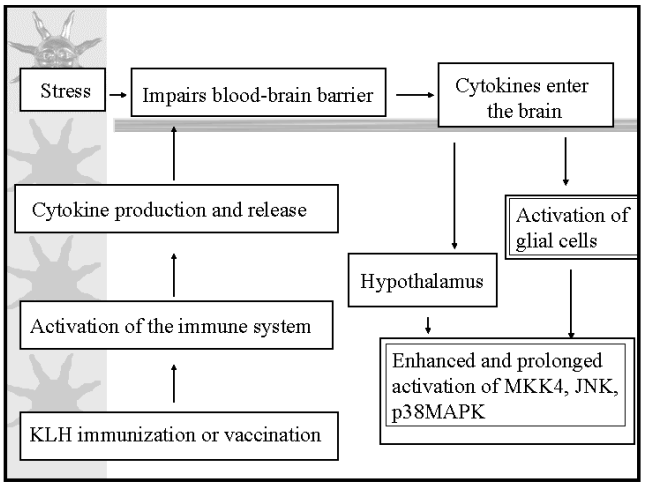


KLH significantly prolongs activation of MKK4 in the brain



KLH immunization in combination with forced-swim stress triggers activation of p38MAPK

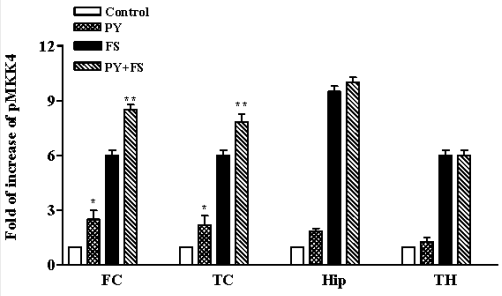


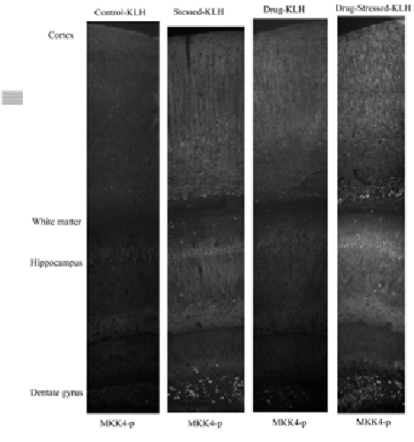


Part V

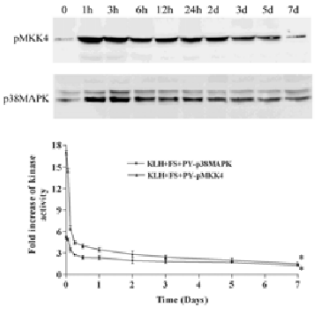
Pyridostigmine (PY)

PY slightly enhances forced-swim stress-induced MKK4 activation





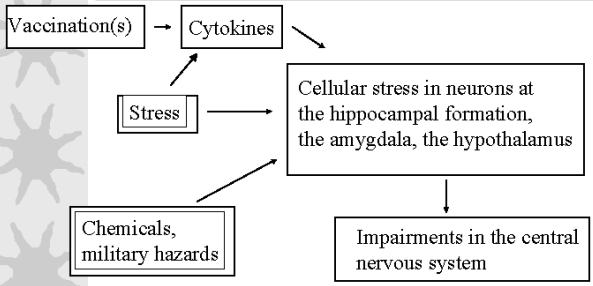
PY further prolongs activation of MKK4 and p38MAPK



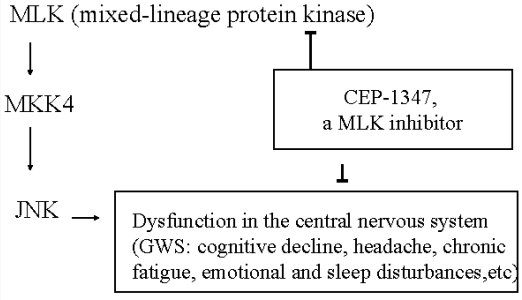
Summary

- Administration of pyridostigmine further promotes and prolongs activation of stress-activated kinases induced by forced-swim stress in combination with KLH immunization.

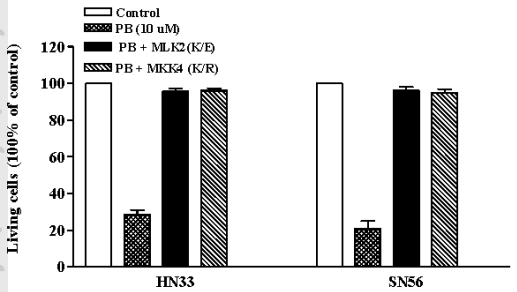
Relationship to GWS



Signaling pathway of stress-activated kinases



Inactivation of MLK2 or MKK4 blocks neuronal toxicity induced by PB



Future Studies

- Effects of other chemicals such as DEET, permethrin;
- Generate chronic stress model;
- Effect of the MLK inhibitor, CEP-1347 on our mouse models
- MLK2 knockout mice

Presentation 7 - Mohan Sopori

Immunotoxicity of low-dose sarin and silica inhalation

Mohan Sopori, PhD
Immunology Division

Lovelace Respiratory Research Institute
Albuquerque, New Mexico, USA

Gulf War Syndrome (GWS)

- Some Gulf War veterans experience symptoms such as mood changes, loss of concentration, chronic fatigue, sleep disturbances, muscle and joint pains, skin rashes, Chronic digestive problems, loss of sex drive.
- Etiology of GWS is unknown. The war-related psychological stress, and exposures to nerve gas, anti-nerve gas prophylactics, insecticides, pathogens, vaccines, silica, etc have been considered to contribute to the symptomatology of the syndrome.

• EFFECTS OF SARIN AND SILICA ON THE IMMUNE SYSTEM

Potential immunological bases of the GWS

- Proinflammatory cytokines such as IL-1, IL-6, and TNF-α play a pivotal role in inflammation and neuroimmune-endocrine interactions.
- These cytokines ("alarm cytokines") are also produced in response to psychological stressors, and administration of high doses induces symptoms similar to those experienced by Gulf War veterans, i.e., arthralgia, headache, skin rash, fevers, decreased appetite appetite and libido, mood alterations, and fatigue.
- Agents such as sarin and silica might modulate the immune and inflammatory systems, leading to cytokine imbalances.

Nerve Gas (sarin) and Gulf war syndrome

- Sarin is a power nerve agent that in high doses may cause seizures and death.
- Its lethality and low cost of production makes it the chemical of choice for terrorism. The 1994 subway sarin attack in Japan, caused many deaths and injured over 6,000 people. Some of the survivors succumbed to Legionella infection.

Cholinergic Toxicity

- Inhibition of acetylcholinesterase (ChE) with organophosphates such as the nerve agent sarin, results in overabundance of acetylcholine (ACh) at the synaptic cleft, which might lead to lethality and convulsive seizures. Moreover, ChE inhibitors may induce psychopathologies that resemble the post-traumatic stress disorder.
- ACh stimulates both muscarinic and nicotinic receptors, and the lethality of anti-cholinesterase toxicity is reduced by cholinergic receptor antagonists together with the ganglionic blockers (e.g., chlorisondamine, hexamethonium), which act on the nicotinic receptors in the autonomic nervous system.
- Does sarin affect the immune system as nicotine?

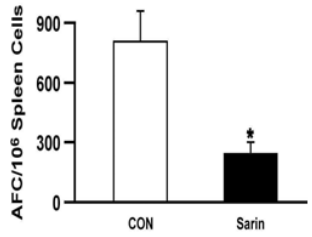
In rats and mice, chronic administration of nicotine suppresses the immune system, and the immunosuppression persists for several weeks, even when nicotine treatment is discontinued.

AFC/10^6 Spleen Cells

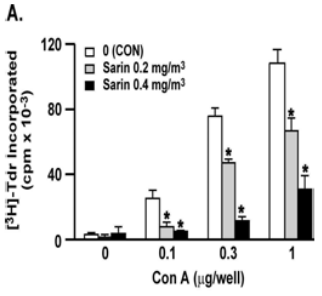
Treatment	n	Antibody-forming cells
Control	5	762 ± 106
Nicotine (4 wk)	4	296 ± 54
Post-nicotine (2 wk)	6	234 ± 70

Animals were implanted subcutaneously (s.c.) with saline (control)- or NT-containing miniosmotic pumps, and 4 days prior to sacrifice, animals were immunized with SRBC. Spleen cells were analyzed for anti-SRBC AFC responses by standard methods (Sopori et al., 1989).

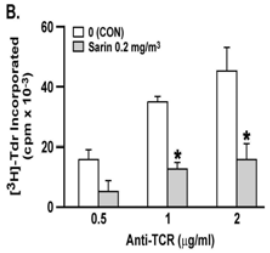
Sarin inhibits antibody response



Sarin suppresses T cell mitogenesis

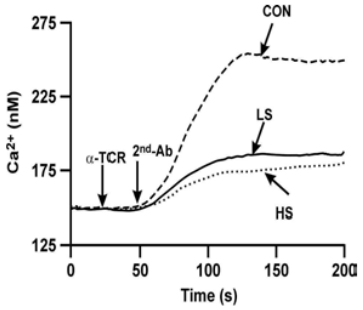


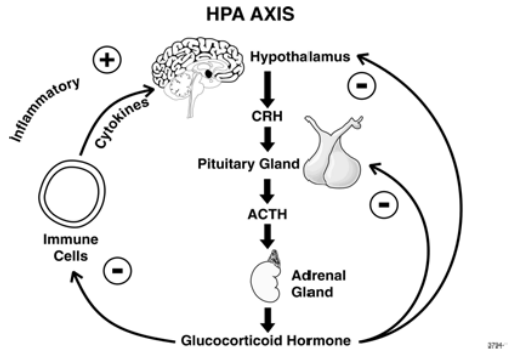
Sarin inhibits antigen-induced T cell proliferation

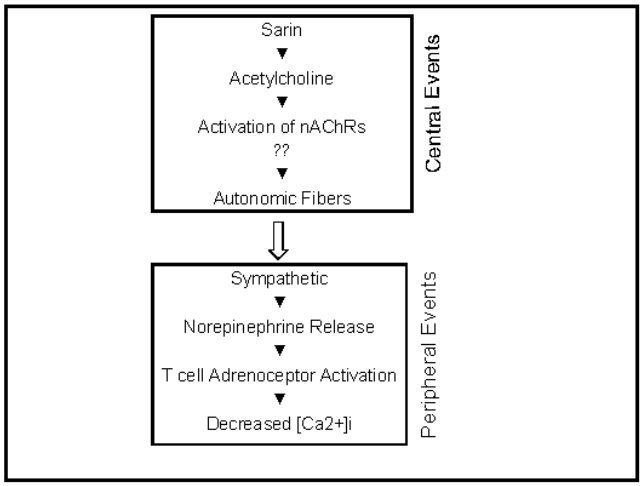


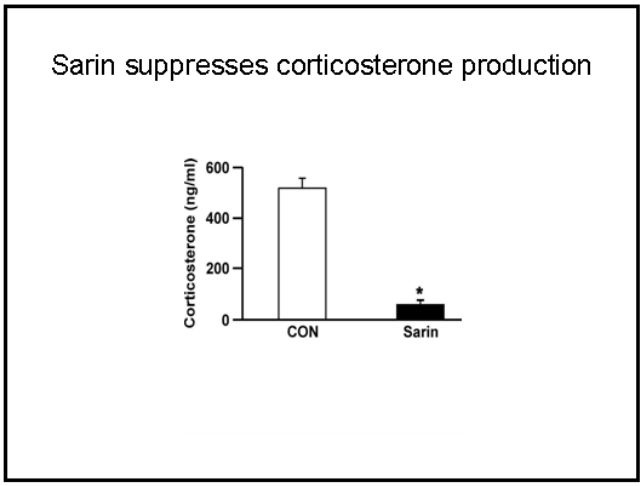
TCR + Ag
↓
Src-like PTK (Fyn)
↓
PTK
↓
Phospholipase C-γ1 (PLC-γ1)
↓
Inositol-1,4,5-trisphosphate (IP3)
↓
Increased $[Ca^{2+}]_i$
↓
G0/G1 → S-phase

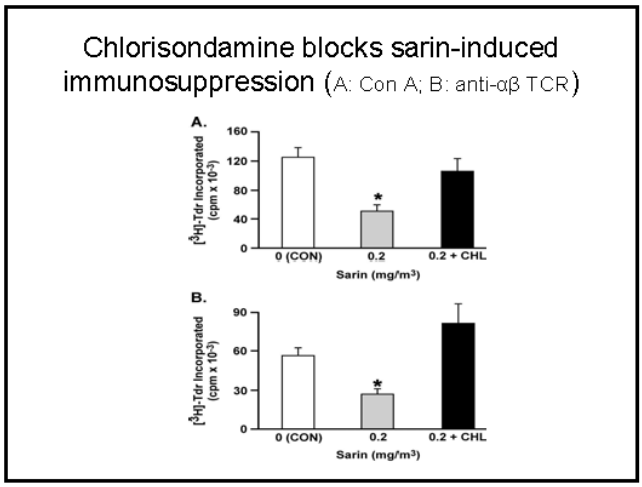
Sarin impairs T cell signaling

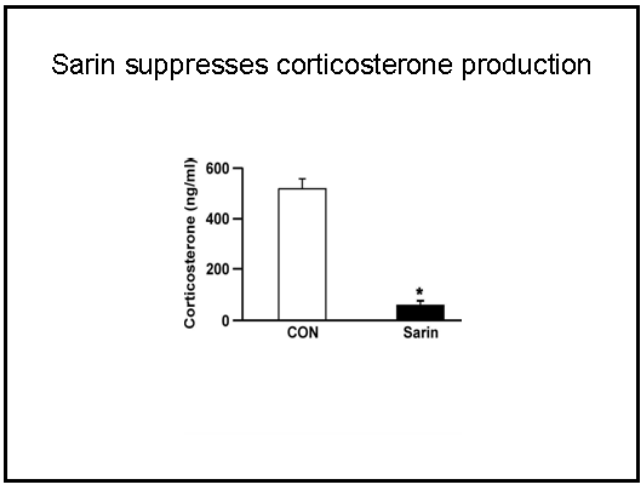




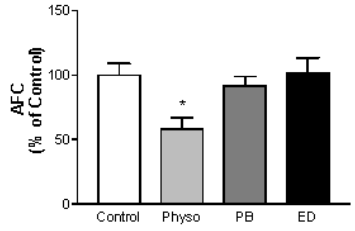

Sarin
Acetylcholine
Activation of nAChRs
??
Autonomic Fibers
Central Events
Sympathetic
Norepinephrine Release
T cell Adrenoceptor Activation
Decreased [Ca2+]i
Peripheral Events


Sarin suppresses corticosterone production
Corticosterone (ng/ml)
600
400
200
0
CON
Sarin

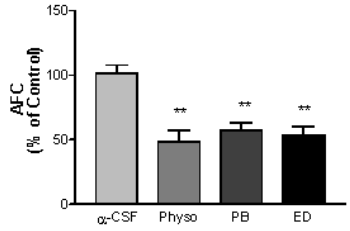

Chlorisondamine blocks sarin-induced immunosuppression (A: Con A; B: anti-αβ TCR)
A.
[3H]-Tdr Incorporated (cpm x 10-3)
160
120
80
40
0
0 (CON)
0.2
0.2 + CHL
Sarin (mg/m3)
B.
[3H]-Tdr Incorporated (cpm x 10-3)
90
60
30
0
0 (CON)
0.2
0.2 + CHL
Sarin (mg/m3)


Sarin suppresses corticosterone production
Corticosterone (ng/ml)
600
400
200
0
CON
Sarin

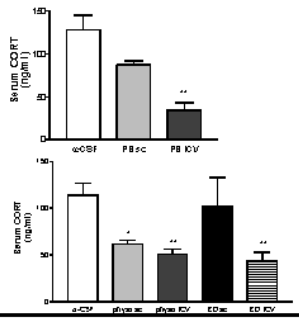
SC administration of physostigmine (0.2 mg/kg), but not pyridostigmine (0.5 mg/kg) or edrophonium (1.5 mg/kg) inhibits the antibody response



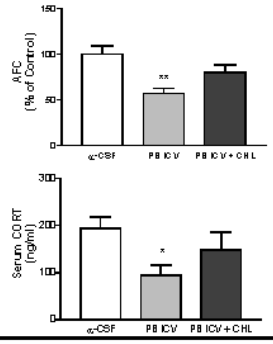
Intracerebroventricular administration of low doses of physostigmine (0.02 mg/kg), pyridostigmine (0.5 mg /kg), or edrophonium (1.5 mg/kg) inhibits the antibody response

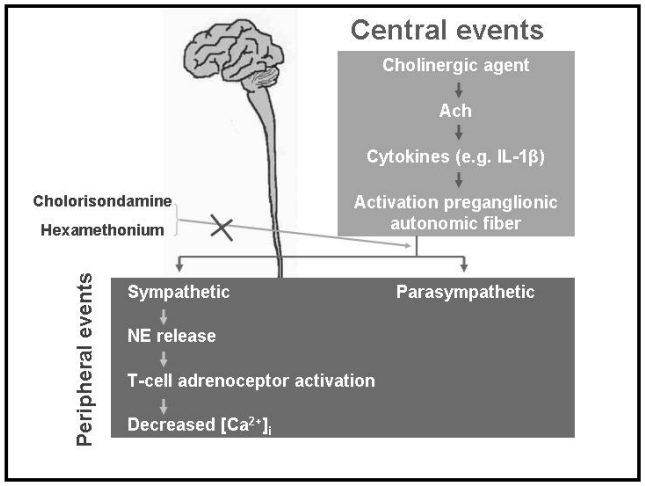


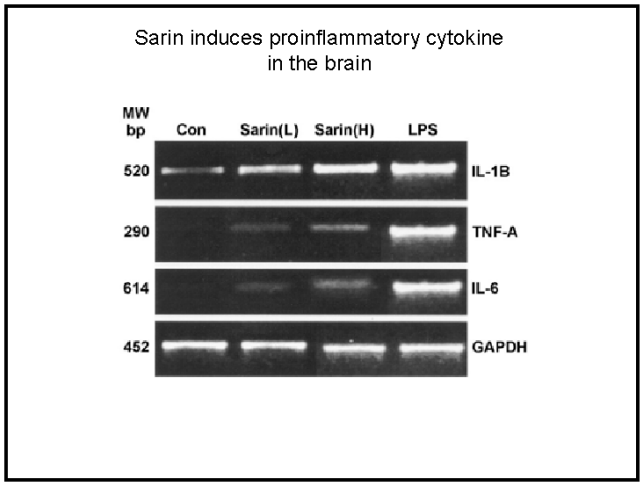
Central administration of cholinergic agents suppresses glucocorticoid production

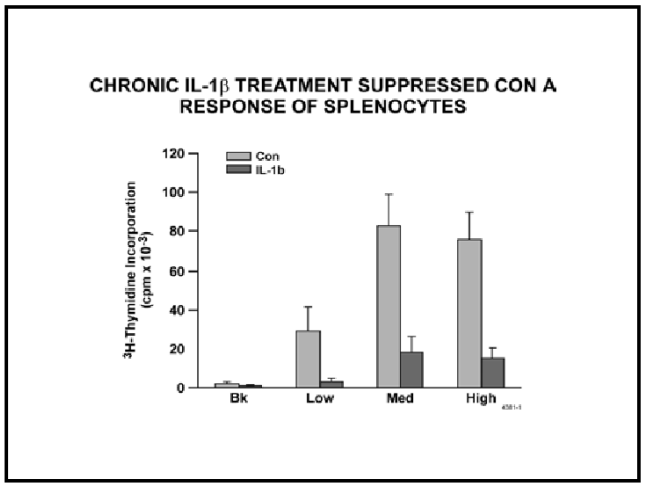


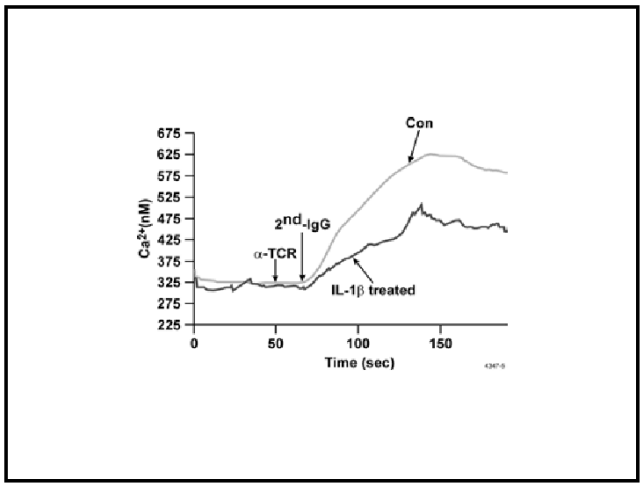
The ganglionic blocker, chlorisondamine (CHL), attenuates the inhibitory effects of pyridostigmine on the antibody and glucocorticoid responses

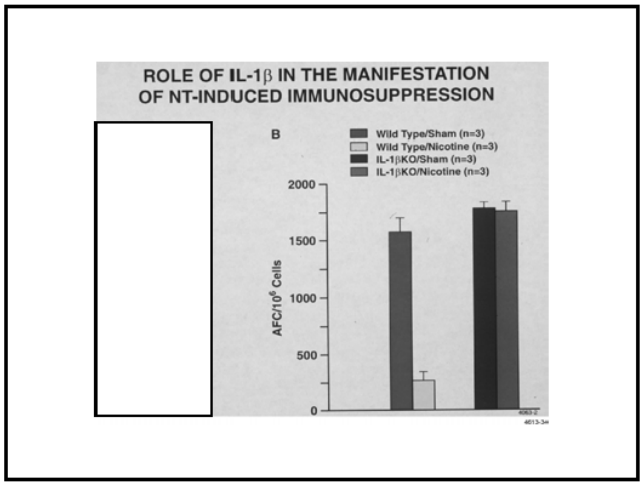


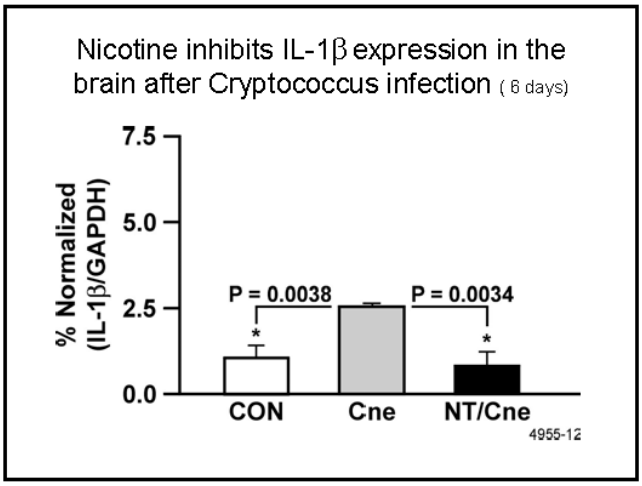

Central events
Cholinergic agent
Ach
Cytokines (e.g. IL-1β)
Activation preganglionic autonomic fiber
Cholorisondamine
Hexamethonium
Peripheral events
Sympathetic
Parasympathetic
NE release
T-cell adrenoceptor activation
Decreased [Ca2+]i

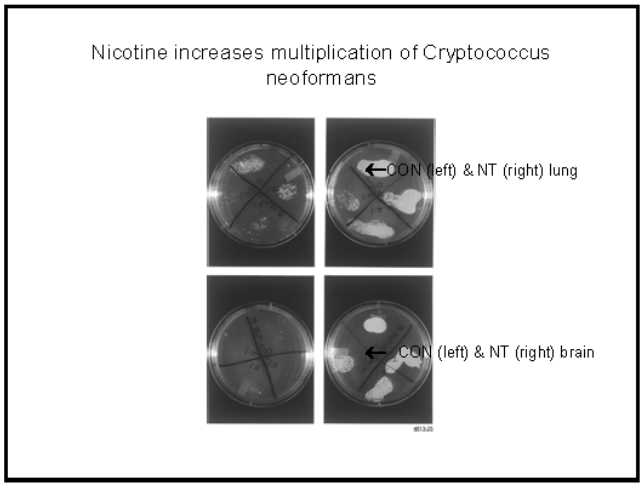

Sarin induces proinflammatory cytokine in the brain
MW bp
Con
Sarin(L)
Sarin(H)
LPS
520
IL-1B
290
TNF-A
614
IL-6
452
GAPDH

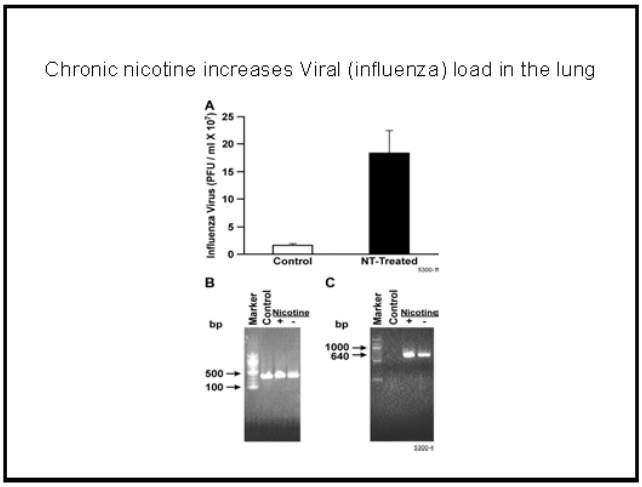

CHRONIC IL-1β TREATMENT SUPPRESSED CON A RESPONSE OF SPLENOCYTES
Con
IL-1b
3H-Thymidine Incorporation (cpm x 10-3)
120
100
80
60
40
20
0
Bk
Low
Med
High


Con
675
625
575
525
475
425
375
325
275
225
Ca2+(nM)
2nd-IgG
α-TCR
IL-1β treated
0
50
100
150
Time (sec)


ROLE OF IL-1β IN THE MANIFESTATION
OF NT-INDUCED IMMUNOSUPPRESSION
B
Wild Type/Sham (n=3)
Wild Type/Nicotine (n=3)
IL-1βKO/Sham (n=3)
IL-1βKO/Nicotine (n=3)
AFC/10^6 Cells
2000
1500
1000
500
0


Nicotine inhibits IL-1β expression in the
brain after Cryptococcus infection (6 days)
% Normalized
(IL-1β/GAPDH)
7.5
5.0
2.5
0.0
P = 0.0038
P = 0.0034
*
*
CON
Cne
NT/Cne
4955-12


Nicotine increases multiplication of Cryptococcus
neoformans
CON (left) & NT (right) lung
CON (left) & NT (right) brain


Chronic nicotine increases Viral (influenza) load in the lung
A
Influenza Virus (PFU / ml X 10^3)
25
20
15
10
5
0
Control
NT-Treated
B
C
bp
Marker
Control
Nicotine
+
-
500
100
1000
640

CONCLUSIONS

- Sarin and other cholinergic agents suppress the immune system.
- Cholinergic agents affect the immune system centrally through the autonomic nervous system.
- Sarin induces IL-β in the brain, and Chronic ICV administration of IL-1β suppresses the immune system. This immunosuppression may increase the risk for infections.
- Sarin and other cholinergic agents inhibit glucocorticoid production primarily through the autonomic nervous system. Increased plasma cortisol levels might serve as a marker for cholinergic exposure.

SILICA AS AN IMMUNOMODULATOR

- Saudi Arabian sand is rich in silica and contains a significant fraction of ultra-fine particles and microbial material.
- Silica is an adjuvant and, in susceptible individuals, might induce lung granulomas and autoimmune disorders.

SILICOSIS

- Silicosis is usually associated with occupational exposure to crystalline silica.
- Silicosis is usually diagnosed many years after the SL exposure; thus, the disease might progress over many years after the exposure has ended.
- In most experimental silicosis, animals show rapid lung inflammation and injury.

Methods

- Most prevalent animal models of silicosis use IT administration of large silica doses, leading to acute lung injury (apoptosis).
- Rats were exposed to silica inhalation (5-6 mg/m3, 6 h/d, 5 d/wk for 6 wk).
- We determined the immunological and inflammatory status of these animals.
- **CONCLUSION:** Acute silicosis may be mechanistically different from chronic silicosis.

Tissue silica burden (ng/mg) at the end (0 time) and 10 wk after silica treatment¶

Time§	Lung	Spleen	Brain	Liver
0 Time				
SL	413 ± 59	708 ± 154	186 ± 48	ND
10 Weeks				
SL	111 ± 8	51 ± 3	38 ± 3	50 ± 13

[a] SL, silica-treated; ND, not done.
[b] Represents time after SL exposure.
[c] Values represent mean ± SEM from five animals/group.

Histopathological changes in the lung and BALT at various times after silica exposure¶

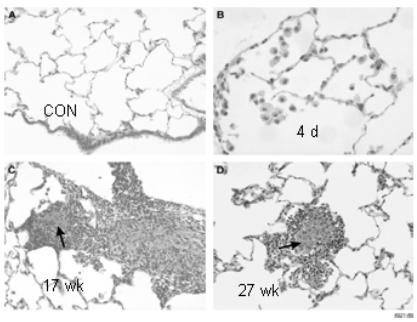
	Lung		
Sacrifice time	Focal AM§	Alveolar PMN/LYMP§	Granuloma
Day 4	0.4	0	0
4 wk	0.8	0	0
10 wk	1.4	1.6	0
17 wk	3.0	2.8	0.4
27 wk	2.5	2.5	2.0

¶Numerical values represent average grade from five animals/group.

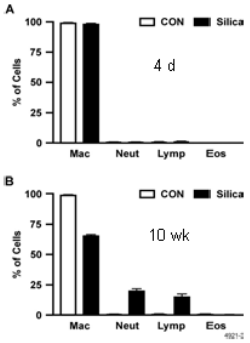
Silica increases protein and LDH content of BALF at later stages

Time§	LDH (U/L)	Protein (mg/DL)
4 days		
CON	38.0 ± 4.0[c]	2.0 ± 0.3
SL	44.0 ± 3.0	2.3 ± 0.2
10 weeks		
CON	42.0 ± 3.5	2.2 ± 0.4
SL	47.0 ± 4.0	2.8 ± 0.5
17 weeks		
CON	23.4 ± 2.0	2.4 ± 0.4
SL	100.6 ± 24.0 *	6.4 ± 0.7 **

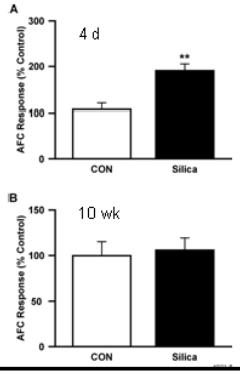
* P ≤ 0.05; ** P ≤ 0.005.



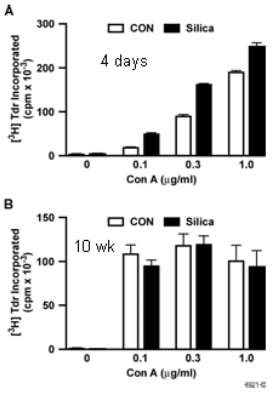
Accumulation of leukocytes in the lung occurs in the later phase of chronic silicosis



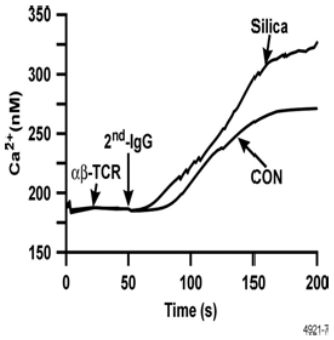
Silica activates antibody response in the early phase of silicosis



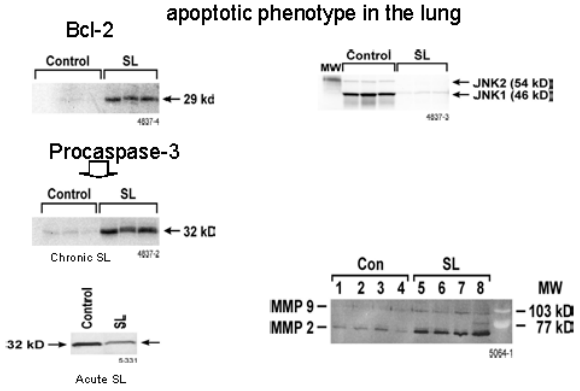
Silica stimulates Con A response in the early phase



Low-dose chronic silica stimulates the antigen-induced rise in the intracellular calcium level in T cells in the early phase of silicosis



Chronic low-dose silica induces anti-apoptotic phenotype in the lung



CONCLUSION

- In susceptible individuals, amount of silica required to induce silicosis may be small.
- Unlike acute silicosis, chronic silicosis shows a delayed inflammatory response and the granuloma formation is associated with anti-apoptotic phenotype.
- At present, the mechanism and the biological consequences of silica accumulation in the brain is unclear.

U.S. Army Medical Research and Materiel Command (DAMD 17-97 & DAMD 17-00-1-0073) NIH (RO1-DA04208-12 & R01-DA04208S-7)

Roma Kalra
Ray Langley
Neerad Mishra
Juan C. Philippides
Seddi Razani
Shashi Singh
Rogene Henderson
Fletcher Hahn
Tom March

Inhalation Department
Ed Barr (Dir. Inhal. Facility)

Presentation 8 - John Ottenweller

Immune Dysregulation in Gulf Veterans with CFS and its Relationship with Cognitive Function and Functional Status

William Gause, Gudrun Lange,
John Ottenweller and Benjamin Natelson

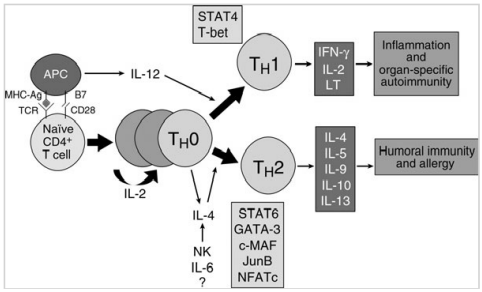
NJ Environmental Hazards Research Center and
War-Related Illness and Injury Study Center

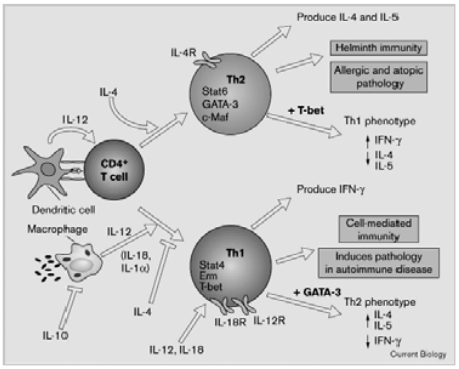
Background of our Immune Studies

- Hypothesis that CFS is due to infection and immune dysregulation.
- We have identifed CFS in GVs, and it is the only medical condition that occurs more frequently in GVs than non-Gulf era veterans.
- We examined the hypothesis that immune function is dysregulated in GVs with CFS.

1997 Rook and Zumla paper

- Hypothesis: GWI due to a switch from Th1 to Th2 profile
  - Associated with EBV reactivation
  - Vaccines with large antigenic load
    - Those given troops going to Gulf
  - Th2 particularly responsive to stress
  - Exposure to insecticides inhibits Th1 IL-2 functions
- Recent Paper by Peakman, Wessely and their colleagues shows elevated Th2-like cells in civilians with CFS





Hypothesis #1

- GVs with CFS show an up-regulated Th2 immune profile.
  - **Alternative that Th1 cytokines will be elevated**
- Differentiation to Th2 cells due to IL-4 and IL-6
- Th2 cells secrete IL-4, IL-5, IL-9, IL-10 and IL-13

Demographics for GVs

	Healthy	CFS
Total n =	34	43
Male	88%	74%
White	85%	77%
Education		
> high school	79%	56%
Axis I	18%	72%

Methodology

- Collected blood by venipuncture
- PBLs labelled with cell surface markers and counted by FACscan to give cell counts for different types of lymphocytes and their % of the total lymphocytes
- mRNA isolated from PBLs and semiquantitative RT-PCR used to estimate mRNA levels of cytokines

Immune Variables

- $CD3^+$ (Total T Cells)
- $CD3^+CD4^+$ (MHC II T Cells)
- $CD3^+CD8^+$ (MHC I T Cells)
- $CD3^-CD19^+$ (B Cells)
- $CD3^-CD[16^+56^+]$ (NK Cells)

- IL-2
- IL-4
- IL-6
- IL-10
- IL-12
- TNF-α
- INF-γ

Cell Types and Cytokines in GWVs

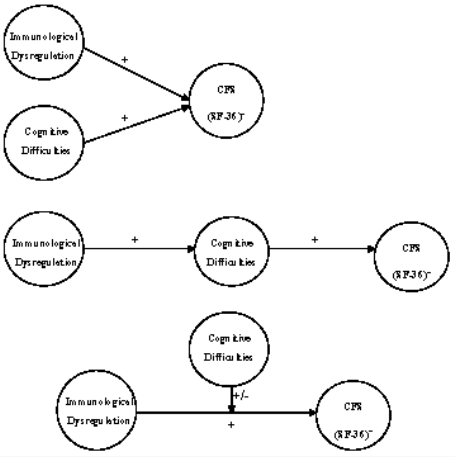
Cell Type	Healthy	CFS	P values
Lymphocytes (Counts)	1918 +/- 96	2120 +/- 104	NS
NK Cells, CD16+56+ (%)	15.5 +/- 1.1	12.2 +/- 0.9	P < 0.02
Total T Cells, CD3+ (%)	71.9 +/- 1.2	76.4 +/- 0.9	P < 0.01
MHC II Cells, CD3+ CD4+ (%)	42.4 +/- 1.4	48.3 +/- 1.0	P < 0.001
Cytokine mRNA			
IL-2	252 +/- 61	431 +/- 140	P < 0.05
IL-4	134 +/- 19	256 +/- 58	NS
IL-6	1711 +/- 337	2882 +/- 505	NS
IL-10	496 +/- 265	604 +/- 137	P < 0.02
IL-12	136 +/- 39	300 +/- 85	NS
INF-gamma	166 +/- 27	289 +/- 48	P < 0.02
TNF-alpha	632 +/- 146	1002 +/- 164	P < 0.01

Summary

- Partially supports shift to Th2 phenotype because IL-10 mRNA is elevated
- However, elevated IL-2 and IFN- γ suggest that Th1 lymphocytes are also activated in GWs with CFS

Hypotheses 2 & 3

- GVs with CFS would be more likely to show cognitive difficulties than the healthy controls.
- The impact of immune up-regulation on CFS caseness would be either independent of, mediated or moderated by cognitive difficulties.



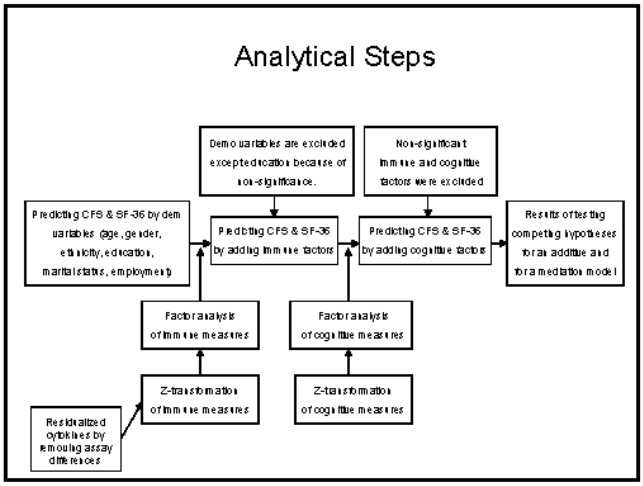
11 Cognitive Tests

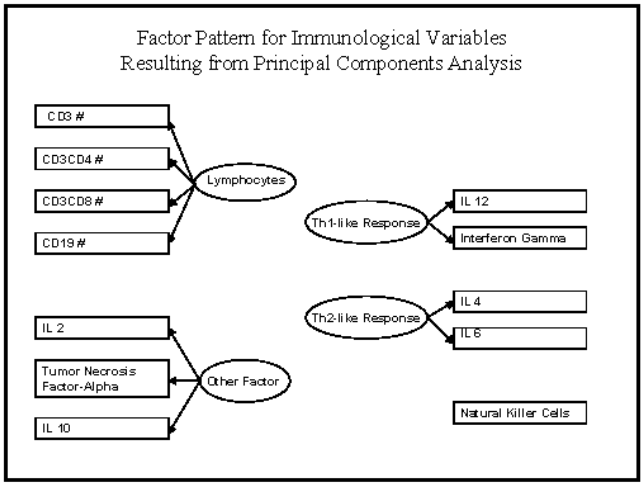
- NES simple and complex reaction time tests
- Paced Auditory Serial Addition Test (PASAT)
- WAIS-R Digit Span subtest
- Category Test
- Verbal Fluency Test
- Rey-Osterrieth Complex Figure Test

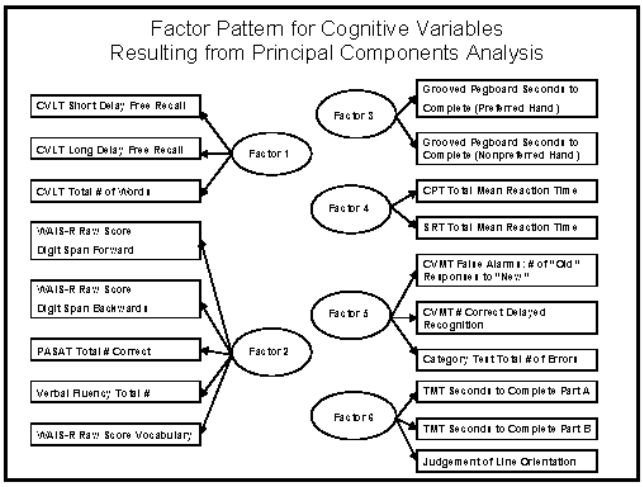
- California Verbal Learning Test
- Continuous Visual Memory Test
- Judgement of Line Orientation
- Wais-R Block Design subtest
- Grooved Pegboard Test

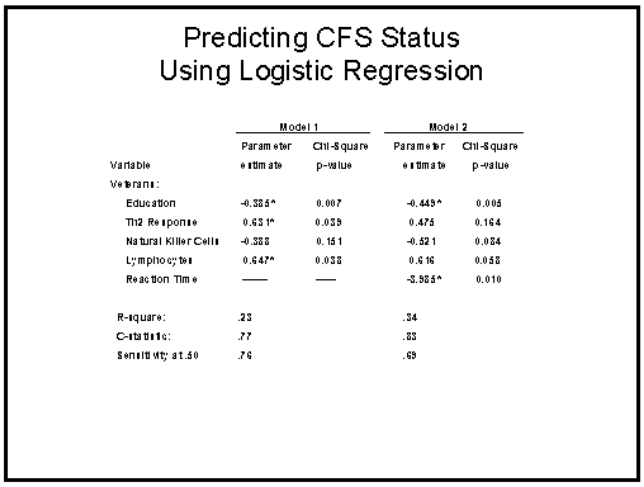
Reasons for using Factor Analysis

- Data reduction in view of small sample size and large number of immune and cognitive variables
- Allows us to see if discrete Th1 and Th2 clusters emerge from raw data









	Model 1		Model 2	
Variable	Parameter estimate	Chi-Square p-value	Parameter estimate	Chi-Square p-value
Veterans:				
Education	-0.535^	0.007	-0.449^	0.005
Th2 Response	0.631^	0.039	0.475	0.164
Natural Killer Cells	-0.338	0.151	-0.521	0.084
Lymphocytes	0.647^	0.038	0.616	0.052
Reaction Time	——	——	-3.935^	0.010
R-square:	.25		.34	
C-statistic:	.77		.83	
Sensitivity at .50	.76		.69	

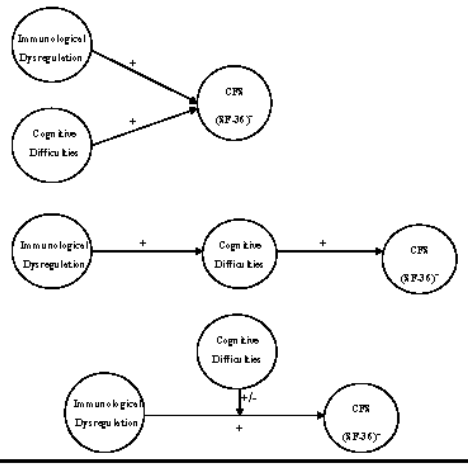
How Do Immune Factors Predict CFS Caseness?

For GVs with CFS, the Th2-like factor and the lymphocyte factor were elevated.
- Th2 result different from first analysis

Supports Rook and Zumla hypothesis that GVs with CFS will exhibit a Type 2 predominance in their cytokines

Controlling for previous predictors, do cognitive factors predict CFS caseness?

- Only one factor -- longer latencies to react -- was associated with CFS caseness
- When reaction time was added to the model, immune factors no longer predict caseness
  - Cognitive problem directly assoc with caseness
  - Immune dysregulation affects cognitive function and is only indirectly associated with caseness



Answers to Hypotheses

- #2: There is a relation between immune dysregulation and CFS for GVs
- #3: This relationship is indirect and mediated by cognitive dysfunction

Hypothesis #4

- The impact of Th2 immune up-regulation on functional status would be either independent of, mediated or moderated by cognitive difficulties.

Predicting Physical Functioning Using MANOVA

Variable	Model 1 Parameter estimate	Model 1 Pr > |t|	Model 2 Parameter estimate	Model 2 Pr > |t|
Veterans:				
Education	0.139^	0.014	0.132^	0.011
Th2 Response	-0.147^	0.035	-0.091	0.378
Natural Killer Cells	0.077	0.428	0.068	0.554
Lymphocytes	-0.303^	0.007	-0.264^	0.018
Reaction Time	—	—	0.324^	0.012

^, p-value < 0.05

Predicting Social Functioning Using MANOVA

Variable	Model 1 Parameter estimate	Model 1 Pr > |t|	Model 2 Parameter estimate	Model 2 Pr > |t|
Veterans:				
Education	0.181^	0.001	0.183^	0.001
Th2 Response	-0.212^	0.004	-0.168	0.144
Natural Killer Cells	-0.002	0.980	-0.007	0.940
Lymphocytes	-0.248^	0.033	-0.238^	0.047
Reaction Time	—	—	0.212^	0.020

^, p-value < 0.05

Predicting General Health Using MANOVA

Variable	Model 1 Parameter estimate	Model 1 Pr > |t|	Model 2 Parameter estimate	Model 2 Pr > |t|
Veterans:				
Education	0.214^	0.000	0.218^	0.000
Th2 Response	-0.184^	0.010	-0.148	0.105
Natural Killer Cells	0.031	0.774	0.033	0.747
Lymphocytes	-0.273^	0.022	-0.288^	0.004
Reaction Time	—	—	0.247	0.068

^, p-value < 0.05

Predicting Mental Health Using MANOVA

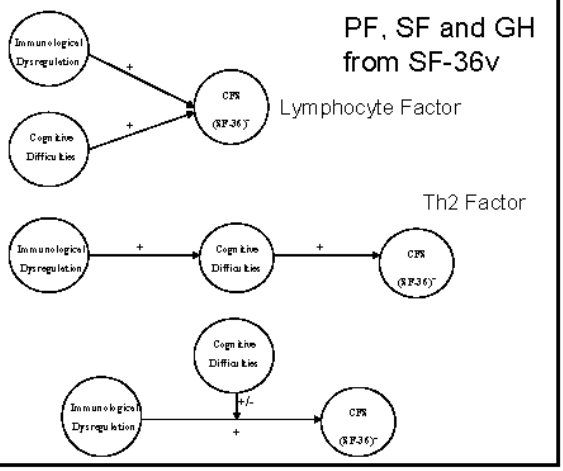
	Model 1		Model 2	
Variable	Parameter estimate	Pr > |t|	Parameter estimate	Pr > |t|
Veterans:				
Education	0.218*	0.002	0.217*	0.001
Th2 Response	-0.163	0.079	-0.043	0.719
Natural Killer Cells	0.120	0.337	0.093	0.448
Lymphocytes	-0.161	0.243	-0.123	0.373
Reaction Time	——	——	0.343*	0.033

* p-value < 0.05

Immune and Cognitive Factors and the SF-36

- Th2-like and lymphocyte factors are associated with poorer general health, and physical and social functioning in GVs
  - Not associated with poorer mental health
- Reaction time, when added to model, predicted functional disability for GVs
- Impact of Th2-like factor on function in GVs was mediated by reaction time
  - Lymphocyte factor remained independent of reaction time

PF, SF and GH from SF-36v



Answers to Hypotheses about Functional Status

- #4: There is a relation between immune dysregulation and functional abilities in GVs
- #4: There is an indirect relationship between the Th2-like factor and functional abilities that is mediated by cognitive dysfunction
  - There is a direct relationship between the lymphocyte factor and functional abilities for GVs

Conclusions

- Mediation model was partially supported in GVs
- Based on factor analyzed immune measures, we found elevated Th2-like and lymphocyte factors in GVs with CFS
  - Supports Rook and Zumla hypothesis
- Effect of elevated immune factors on CFS caseness is mediated by reaction time factor

- Elevated Th2-like factor was indirectly associated with poor functional status in GVs with CFS
  - Effect mediated by reaction time factor
- Elevated lymphocyte factor was directly associated with poor functional status in these veterans
- Reaction time factor was associated with poor functional status for GVs with CFS

National Health Survey of Gulf Era Veterans and their Spouses

- Phase III
  - Representative subsample of NHS Phases I/II
  - Comprehensive exams including H&P, psych evaluation, blood samples, etc.
  - 3250 serum samples
    - About 1000 each from GVs, EVs and Spouses
    - We received 300 microliter aliquots of all samples

Funded Pilot Project

- Measured Cortisol, Paraoxonase and Butyrylcholinesterase in all 3,250 samples
- Completed assays January, 2003
- Statistical analyses of cortisol completed

Cytokine Study in NHS Samples

- Selected totally random samples of 71 GVs and 69 EVs for measurement of plasma cytokine levels
- No consideration of health status or demographics
- Used Luminex technology to measure 9 cytokines simultaneously in 50 microliters serum

Preliminary Plasma Cytokine Levels in 71 Gulf Vets and 69 non-Gulf Era Veterans

Cytokine	Non-Detectable[1]	Published Levels[1]	Overall Levels[2]	EVs*	GVs*
IL-1β	14 (11.4%)	1.1 (0- 44) n=22	13.7 (9.7- 19.7)	17.4 ± 13.0	18.9 ± 29.9
IL-2	11 (7.8%)	12.0 (0-518) n= 9	34.5 (23.2- 87.8)	41.7 ± 45.3	40.7 ± 45.3
IL-4	9 (6.4%)	5.4 (0- 17) n=10	33.2 (23.4- 51.4)	41.8 ± 28.5	33.0 ± 19.9
IL-6	8 (5.7%)	2.0 (0-233) n=30	14.8 (11.1- 27.4)	22.3 ± 17.8	19.8 ± 13.2
IL-8	3 (2.1%)	5.0 (0-224) n=21	18.1 (11.4- 23.2)	31.4 ± 22.5	45.4 ± 29.8
IL-10	8 (5.7%)	3.2 (0- 48) n=24	7.8 (3.2- 11.4)	9.2 ± 9.9	8.3 ± 6.0
IL-12	20 (14.3%)	24.0 (0-234) n=14	39.2 (28.9- 53.8)	58.4 ± 81.1	39.3 ± 31.4
IL-13	Not Assayed	20.0 (1-119) n= 5			
IFN-γ	14 (10.0%)	17.4 (0-388) n=12	111.4 (70.4-130.5)	119.8 ± 74.5	113.1 ± 45.9
TNF-α	14 (10.0%)	4.2 (0- 90) n=32	3.3 (1.4- 5.5)	5.2 ± 7.3	3.7 ± 3.2

[1] Published levels are medians of the reported means or medians for control values, the range is in parentheses, and n = the number of studies consulted. All levels are in pg/ml.
[2] Levels for the 140 Veteran Samples: medians (interquartile ranges).
* Cytokine Levels for EVs (n=69) and GVs (n=71): means ± standard deviations.

Summary

- Plasma Th2 cytokines declined (P's ~ 0.1)
  - IL-4 by 16% and IL-6 by 11%
- A plasma Th1 cytokine declined (P ~ 0.1)
  - IL-12 by 33%
- Not the same as mRNA changes, but earlier studies compared sick and healthy GVs
- In this study, GVs and EVs randomly chosen
- For systemic actions, plasma cytokines are the effectors

Factor Analysis of Plasma Cytokines

Model explained 81% of variance.

- Factor 1 (38% of variance)
  - Th2 Cytokines: TNF, IL-10, IL-4, IL-6
- Factor 2 (26% of variance)
  - Th1 Cytokines: IL-2, IFN
- Factor 3 (17% of variance)
  - Other Cytokines: IL-1, IL-8

Vaccination Rates in GVs and EVs

Vaccination	GVs (%)[1]	EVs (%)[1]	P< [2]	GVs (%)[3]	EVs (%)[3]	P< [4]
Anthrax	43.7	4.7	0.001	44.5	10.3	0.001
Typhoid	60.7	44.3	0.001	54.3	50.0	NS
Botulism	15.4	3.2	0.001	12.7	2.9	0.05
Plague	24.4	15.9	0.001	28.2	22.1	NS
Meningitis	14.5	6.4	0.001	28.2	7.4	0.01
Gamma Globulin	63.4	25.0	0.001	60.6	33.8	0.01

[1] %'s out of approximate 1000 depending on the number who answered each question.
[2] Comparison of rates in columns 2 and 3 using χ^2-tests.
[3] %'s out of 71 GVs and 69 EVs.
[4] Comparison of rates in columns 5 and 6 using χ^2-tests.

Cytokines and Vaccinations

- Anthrax
  - Lower IL-12 (Th1)
- Typhoid
  - Elevated IL-2 and IFN (Th1)

Logistic regression predicting vaccinations, P's <0.05

Summary of Plasma Cytokine Results

- Plasma levels of IL-4, IL-6, IL-12 and TNF may be lower in GVs compare with EVs.
- Plasma cytokines cluster into groups of Th1 and Th2 cytokines.
- Decreased levels of IL-12 and increased levels of IL-2 and IFN may be associated with self-reported vaccinations
- Based on only 7% of the NHS Phase III samples

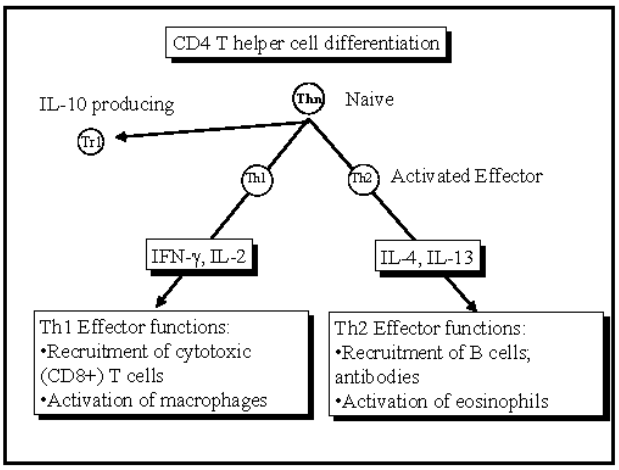
Hypotheses that can be tested with measurement of cytokines in all NHS samples

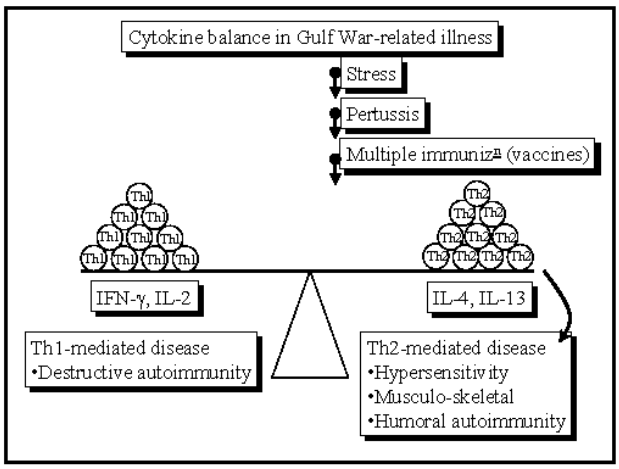
- GVs will have higher levels of Th2 cytokines and lower levels of Th1 cytokines than EVs.
  - Alternatively, Th1 cytokines may be higher in GVs.
- Th2 cytokines will be higher in GVs with poorer physical or mental functioning (SF-36v).
- Th2 cytokines will be higher in GVs with more vaccinations.
- Stress will increase Th2 cytokines and decrease Th1 cytokines.
- Cognitive Impairment will be associated with higher Th2 cytokines in GVs with CMI, but not healthy GVs or EVs.

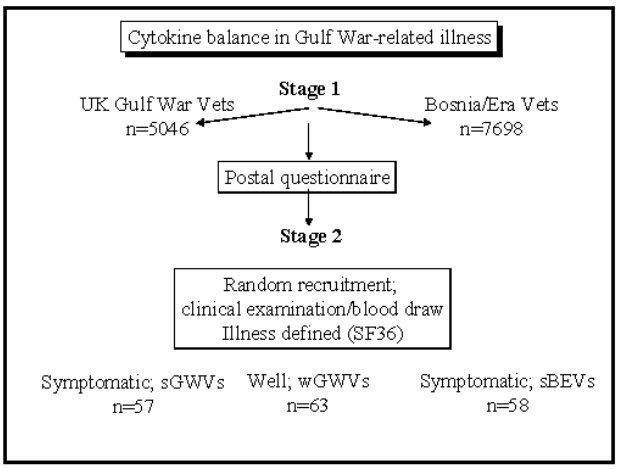
Presentation 9 - Mark Peakman

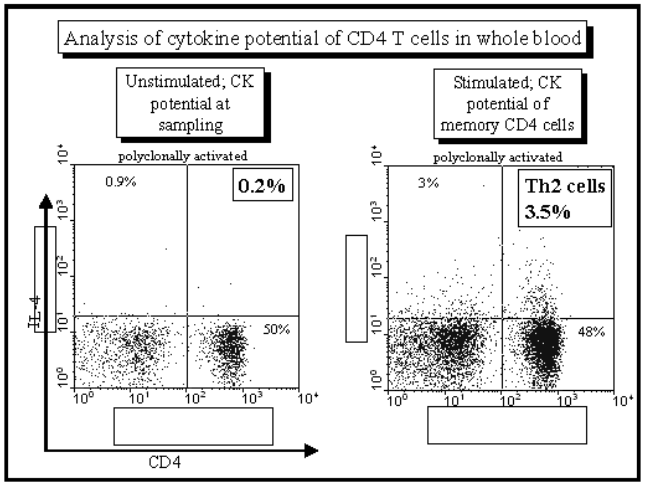
The King's experience

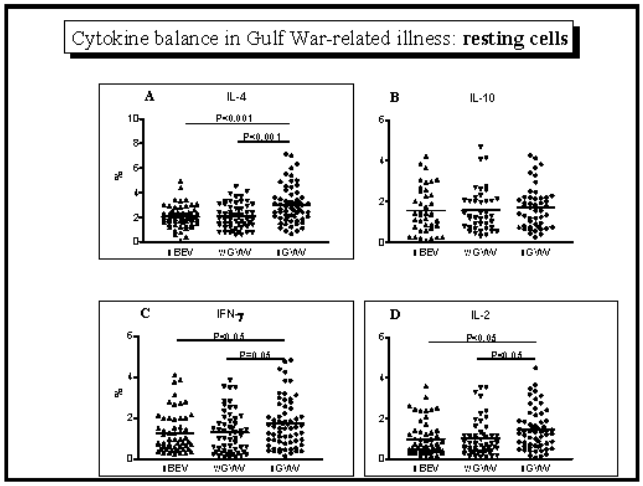
- **Investigating cytokine balance in 1st Persian Gulf War**
  - Epidemiological studies linked multiple vaccines given in theatre of war to multi-symptom illness (Hotopf et al)
  - Rook & Zumla Th1/Th2 hypothesis
- **Investigation of Th1/Th2 hypothesis: direct enumeration of Th1/Th2 cells; autoantibody studies**
- **Investigation of multiple vaccine effects**





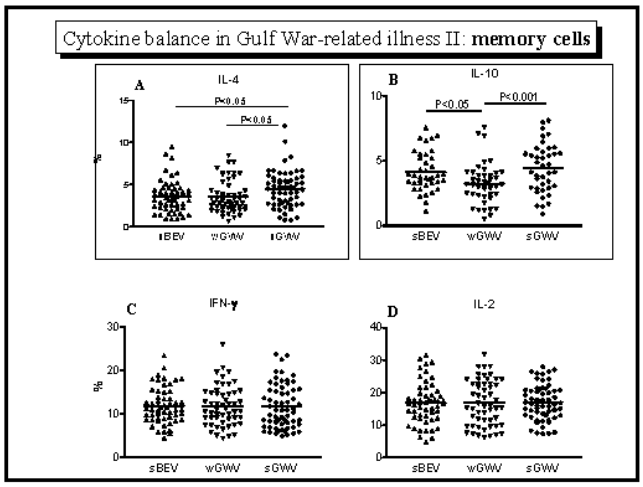






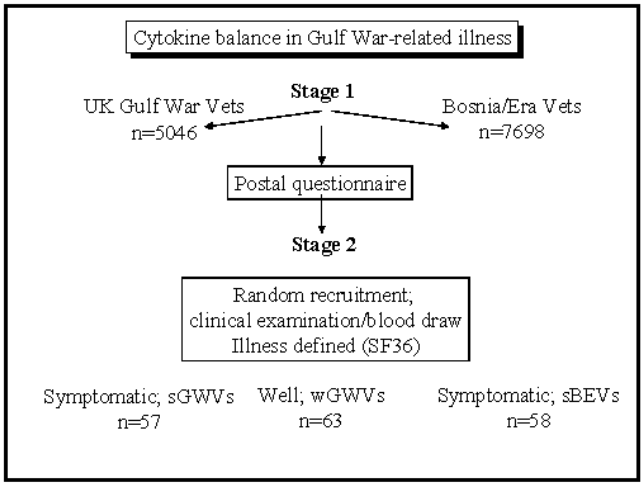
Potential confounders:
Cytokine balance in Gulf War-related illness: **resting cells**

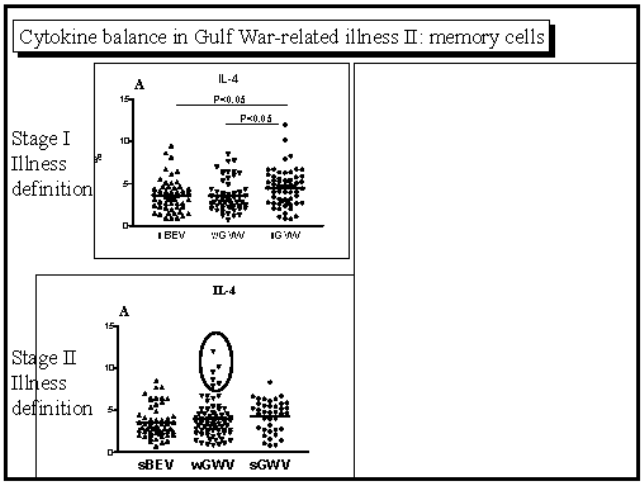
Th type	sGWV (%)	wGWV (%)	Mean diff (corrected for age, sex)	Mean diff (corrected for age, sex, vaccines, depression, atopy)
Th2				
IL-4	3.0	2.4	0.6 **(p=0.04)**	0.3 (p=0.33)
Tr1				
IL-10	1.7	1.6	0.1 (p=0.6)	0.2 (p=0.4)
Th1				
IFN-γ	1.9	1.4	0.5 **(p=0.03)**	0.7 **(p=0.01)**
IL-2	1.6	1.1	0.5 **(p=0.008)**	0.8 **(p=0.001)**



Potential confounders:
Cytokine balance in Gulf War-related illness: **memory cells**

Th type	sGWV (%)	wGWV (%)	Mean diff (corrected for age, sex)	Mean diff (corrected for age, sex, vaccines, depression, atopy)
Th2				
IL-4	4.34	3.6	**1.0 (p=0.05)**	0.7 (p=0.1)
Tr1				
IL-10	4.5	3.2	**1.2 (p=0.003)**	**1.0 (p=0.03)**
Th1				
IFN-γ	11.6	11.9	0.4 (p=0.7)	1.6 (p=0.1)
IL-2	17.3	16.7	1.2 (p=0.3)	1.7 (p=0.2)

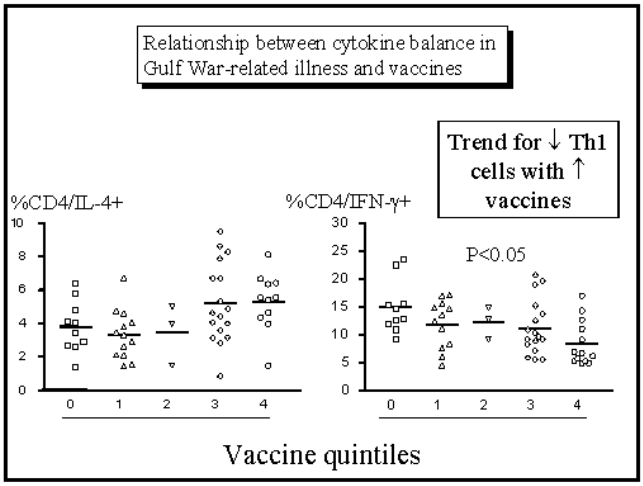




Cytokine balance in Gulf War-related illness: **memory cells (Stage II analysis)**

Th type	sGWV (%)	wGWV (%)	Mean diff (corrected for age, sex)	Mean diff (corrected for age, sex, vaccines, depression, atopy)
Th2				
IL-4	4.3	3.9	0.4 (p=0.3)	0.4 (p=0.4)
Tr1				
IL-10	5.1	3.2	**1.8 (p<0.001)**	**1.9 (p<0.001)**
Th1				
IFN-γ	12.0	11.6	0.6 (p=0.6)	1.6 (p=0.1)
IL-2	17.4	16.4	1.2 (p=0.3)	1.8 (p=0.2)

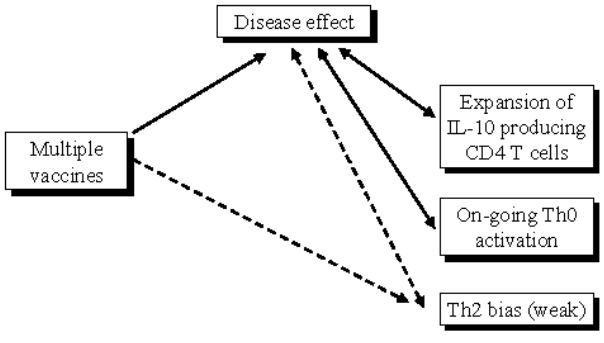
Relationship between cytokine balance in Gulf War-related illness and vaccines



Conclusions I

- **CD4 T cell cytokine balance abnormal in GW-related illness**
  - On-going Th0 (Th1>Th2) activation
  - ↑ IL-10 production by memory cells
  - Th2 activity:- results equivocal
  - Relationship to vaccines: complex

Summary of Th1/Th2 analyses: can we close the loop?



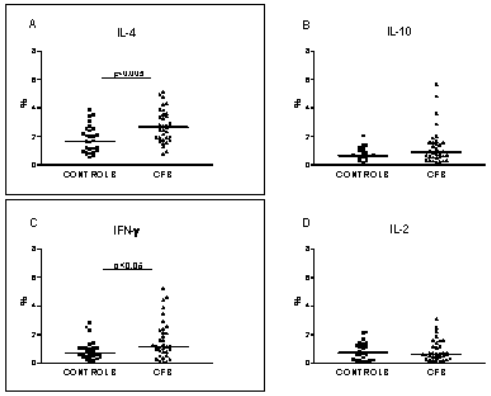
Conclusions II

Expansion of memory IL-10 producing cells:

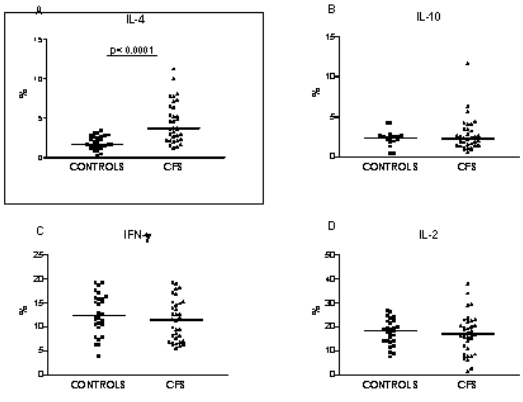
- IL-10 is a major immunoregulatory cytokine
- Inhibits activation and function of T cells and APCs
- Potent negative regulator of Th1 cells
- Mechanisms of IL-10 effect?
- Consequences of IL-10 production for vaccination?

Comparison with chronic fatigue syndrome patients

Cytokine producing cells in chronic fatigue syndrome: **resting CD4**



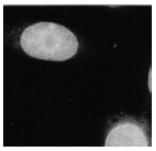
Cytokine producing cells in chronic fatigue syndrome: **memory CD4**



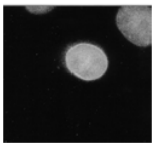
Other potential markers of "Th2-ness":

Analysis of anti-nuclear autontibodsies in GW veterans and CFS patients

Homogenous ANA



Nuclear Envelope (NE) ANA
(Eng Tan, Scripps, 1996)



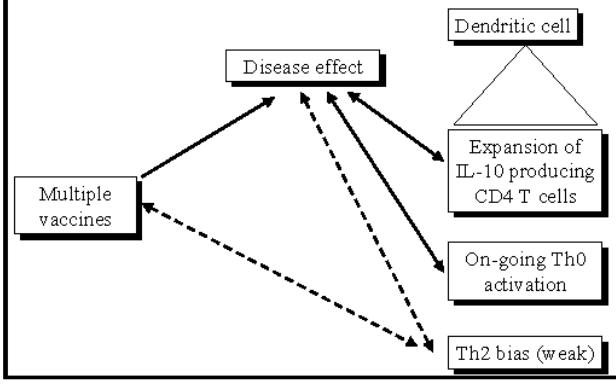
Prevalence of anti-nuclear autoantibodies in Gulf War-related illness

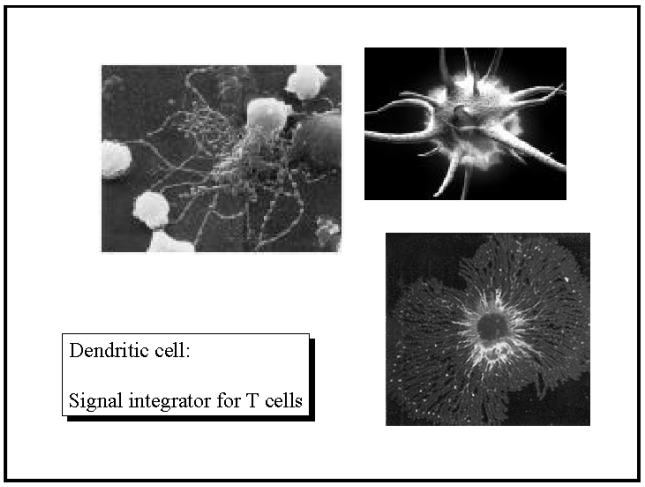
	Homog	Speckled	Nucleolar	Total (%)
sGWV (n=130)	4	3	3	10 (8)
wGWV (n=90)	0	1	2	3 (3)
sBEV (n=128)	7	2	2	11 (9)
Controls (n=51)	2	2	2	6 (12)
CFS (n=100)	0	9	9	18 (18)
Controls (n=111)	7	6	5	18 (16)

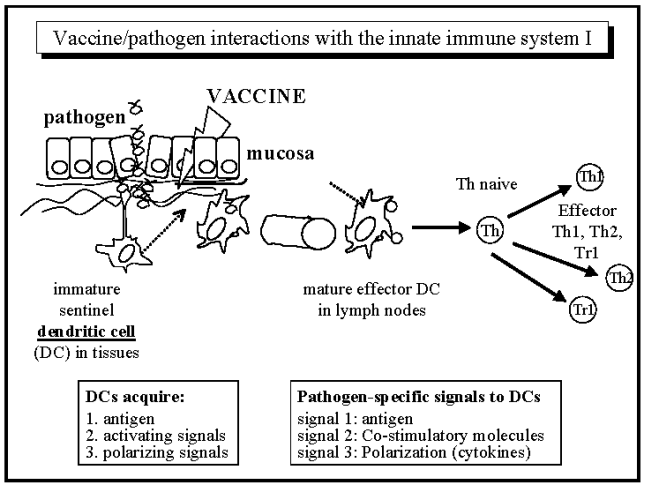
Conclusions III

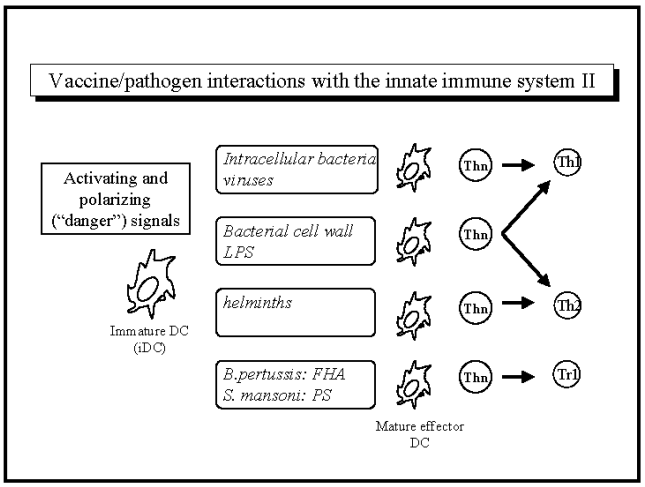
- **There is some resemblance between the Th1/Th2 profile in GW veterans and CFS patients**
  - If primary, suggests common pathogenesis
  - Immune changes could be secondary
- **No evidence for increased anti-nuclear autoimmunity in GW veterans**

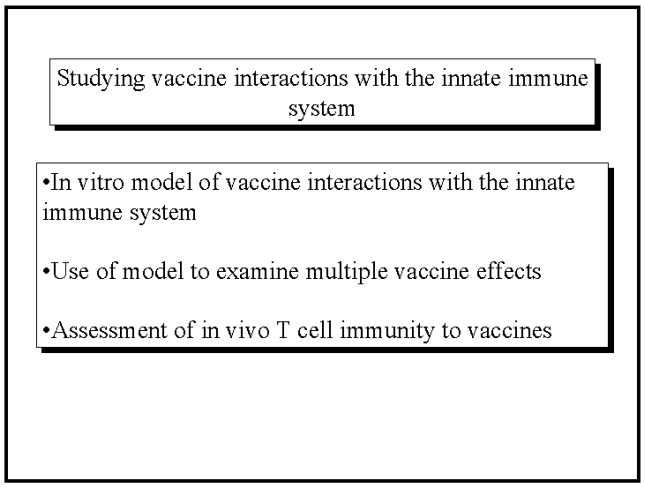
Summary of Th1/Th2 analyses: can we close the loop?

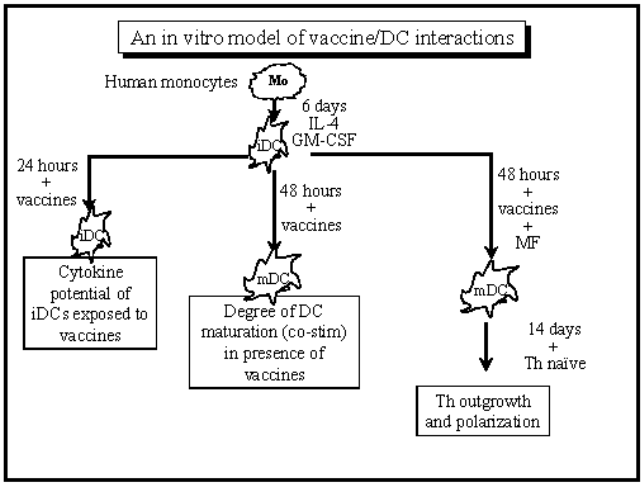


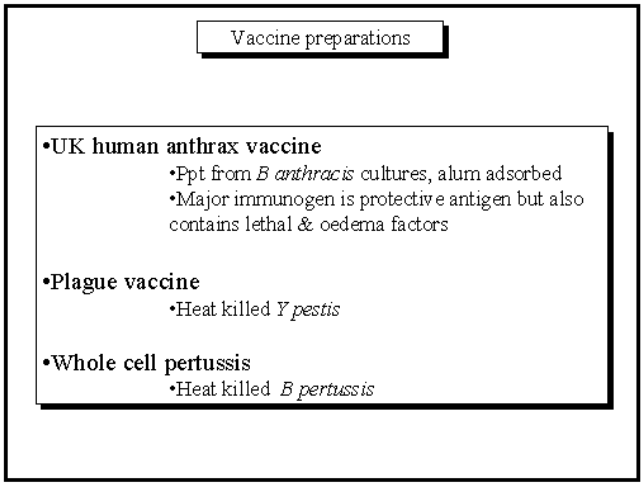

Dendritic cell:
Signal integrator for T cells

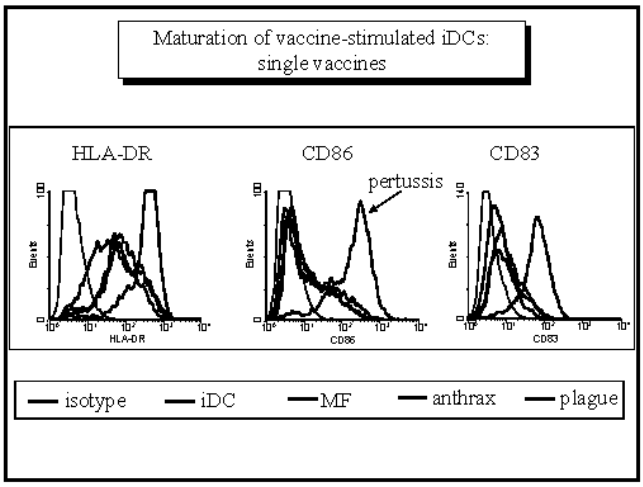

Vaccine/pathogen interactions with the innate immune system I
VACCINE
pathogen
mucosa
Th naive
Th
Th1
Effector
Th1, Th2,
Trl
Th2
Trl
immature
sentinel
dendritic cell
(DC) in tissues
mature effector DC
in lymph nodes
DCs acquire:
1. antigen
2. activating signals
3. polarizing signals
Pathogen-specific signals to DCs
signal 1: antigen
signal 2: Co-stimulatory molecules
signal 3: Polarization (cytokines)

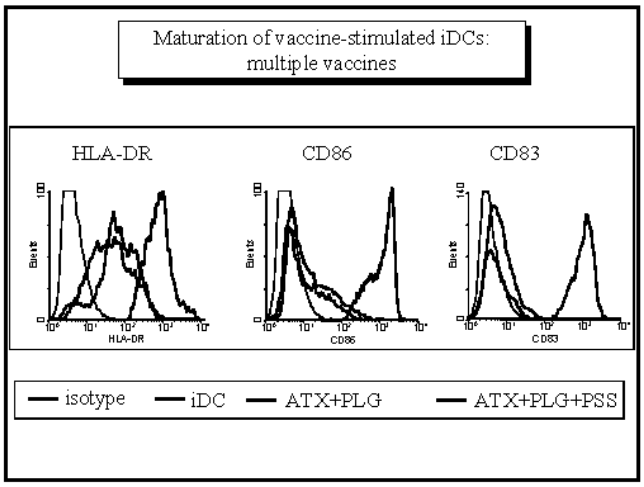

Vaccine/pathogen interactions with the innate immune system II
Activating and
polarizing
("danger") signals
Immature DC
(iDC)
Intracellular bacteria
viruses
Bacterial cell wall
LPS
helminths
B.pertussis: FHA
S. mansoni: PS
Thn
Th1
Th2
Trl
Mature effector
DC

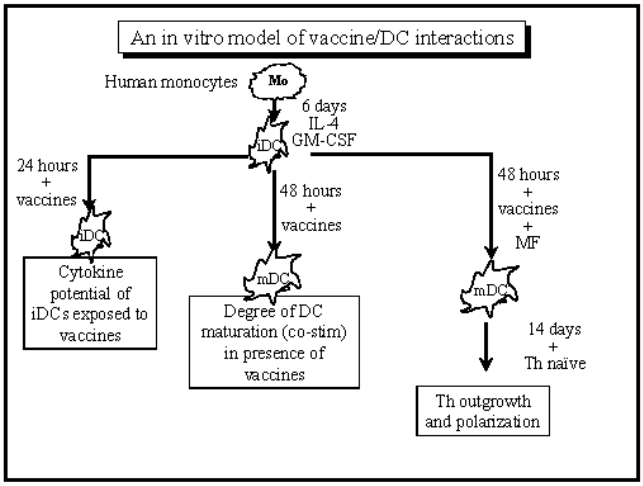

Studying vaccine interactions with the innate immune system
•In vitro model of vaccine interactions with the innate immune system
•Use of model to examine multiple vaccine effects
•Assessment of in vivo T cell immunity to vaccines

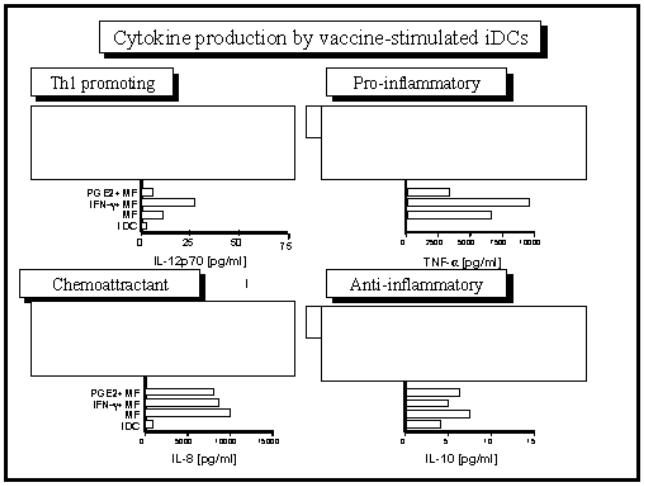

An in vitro model of vaccine/DC interactions
Human monocytes
Mo
6 days
IL-4
GM-CSF
iDC
24 hours
+
vaccines
48 hours
+
vaccines
48 hours
+
vaccines
+
MF
iDC
mDC
mDC
Cytokine potential of iDCs exposed to vaccines
Degree of DC maturation (co-stim) in presence of vaccines
14 days
+
Th naïve
Th outgrowth and polarization

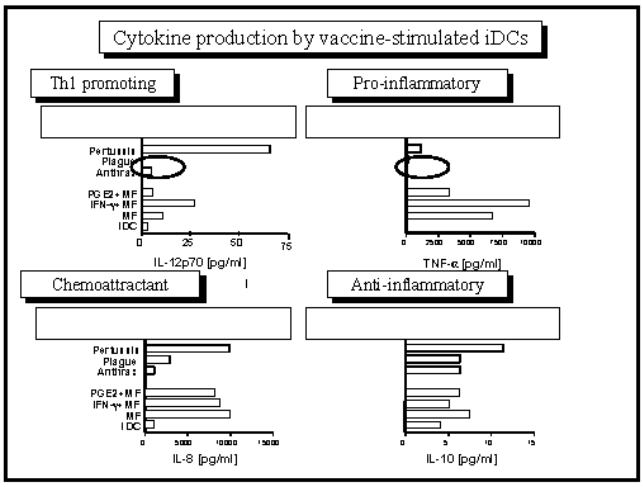

Vaccine preparations
•UK human anthrax vaccine
•Ppt from B anthracis cultures, alum adsorbed
•Major immunogen is protective antigen but also contains lethal & oedema factors
•Plague vaccine
•Heat killed Y pestis
•Whole cell pertussis
•Heat killed B pertussis

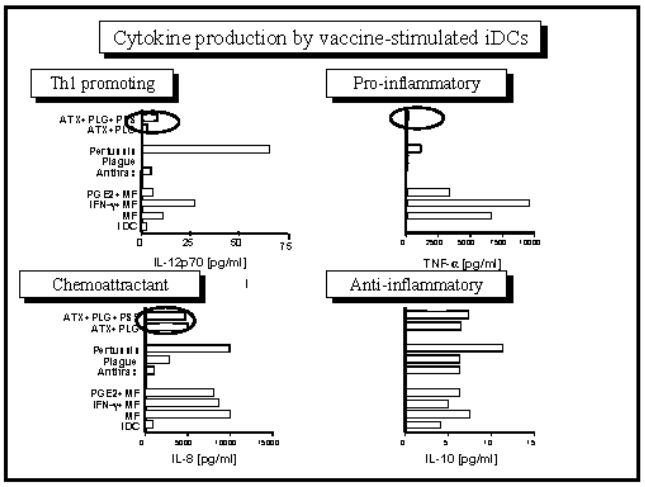

Maturation of vaccine-stimulated iDCs: single vaccines
HLA-DR
CD86
CD83
pertussis
isotype
iDC
MF
anthrax
plague

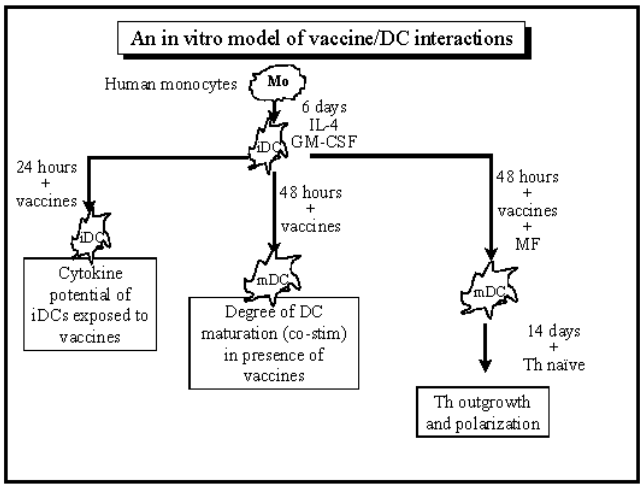

Maturation of vaccine-stimulated iDCs: multiple vaccines
HLA-DR
CD86
CD83
isotype
iDC
ATX+PLG
ATX+PLG+PSS

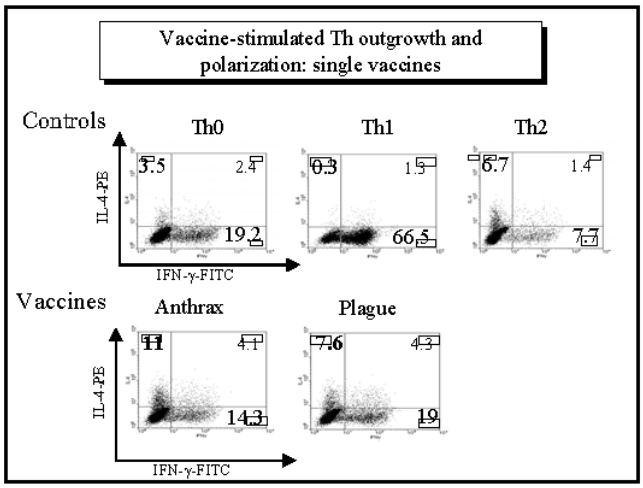

An in vitro model of vaccine/DC interactions
Human monocytes
Mo
6 days
IL-4
GM-CSF
iDC
24 hours
+
vaccines
48 hours
+
vaccines
48 hours
+
vaccines
+
MF
iDC
mDC
mDC
Cytokine potential of iDCs exposed to vaccines
Degree of DC maturation (co-stim) in presence of vaccines
14 days
+
Th naïve
Th outgrowth and polarization

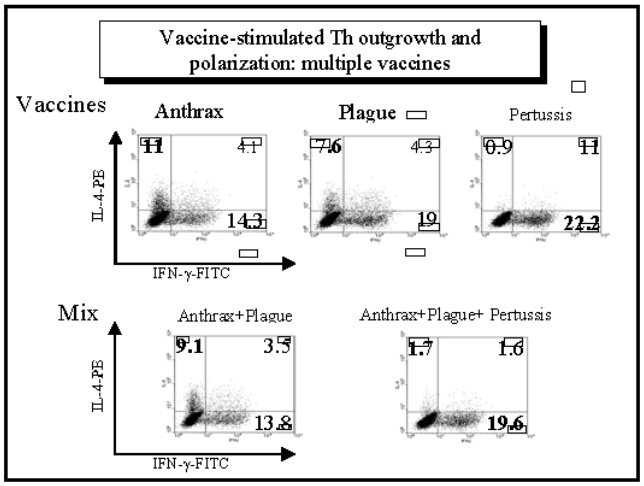

Cytokine production by vaccine-stimulated iDCs
Th1 promoting
Pro-inflammatory
PGE2+MF
IFN-γ+MF
MF
IDC
IL-12p70 [pg/ml]
TNF-α [pg/ml]
Chemoattractant
Anti-inflammatory
IL-8 [pg/ml]
IL-10 [pg/ml]

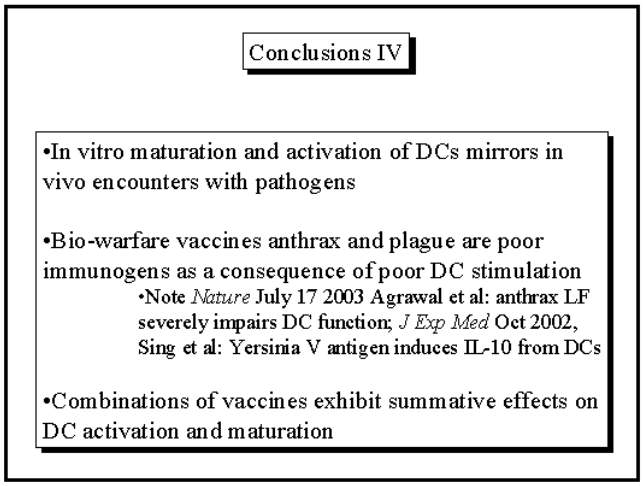

Cytokine production by vaccine-stimulated iDCs
Th1 promoting
Pro-inflammatory
Pertussis
Plague
Anthrax
PGE2+MF
IFN-γ+MF
MF
IDC
IL-12p70 [pg/ml]
TNF-α [pg/ml]
Chemoattractant
Anti-inflammatory
IL-8 [pg/ml]
IL-10 [pg/ml]


Cytokine production by vaccine-stimulated iDCs
Th1 promoting
Pro-inflammatory
ATX+PLG+PS
ATX+PLG
Pertussis
Plague
Anthrax
PGE2+MF
IFN-γ+MF
MF
IDC
IL-12p70 [pg/ml]
TNF-α [pg/ml]
Chemoattractant
Anti-inflammatory
IL-8 [pg/ml]
IL-10 [pg/ml]

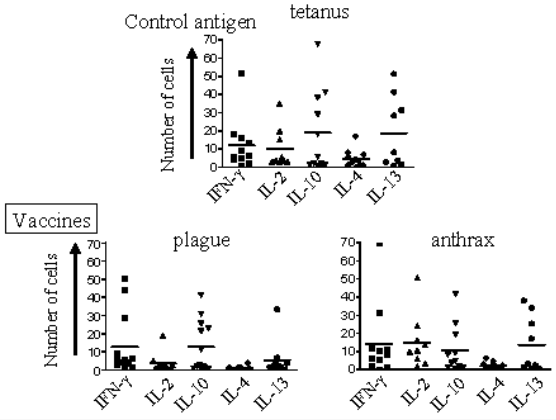

An in vitro model of vaccine/DC interactions
Human monocytes
Mo
6 days
IL-4
GM-CSF
iDC
24 hours
+
vaccines
48 hours
+
vaccines
48 hours
+
vaccines
+
MF
iDC
mDC
mDC
Cytokine potential of iDCs exposed to vaccines
Degree of DC maturation (co-stim) in presence of vaccines
14 days
+
Th naive
Th outgrowth and polarization


Vaccine-stimulated Th outgrowth and polarization: single vaccines
Controls
Th0
Th1
Th2
3.5
2.4
19.2
0.3
1.5
66.5
6.7
1.4
7.7
IL-4-PE
IFN-γ-FITC
Vaccines
Anthrax
Plague
1.1
4.1
14.3
7.6
4.3
19
IL-4-PE
IFN-γ-FITC


Vaccine-stimulated Th outgrowth and polarization: multiple vaccines
Vaccines
Anthrax
Plague
Pertussis
1.1
4.1
14.3
7.6
4.3
19
0.9
1.1
22.2
IL-4-PE
IFN-γ-FITC
Mix
Anthrax+Plague
Anthrax+Plague+ Pertussis
9.1
3.5
13.8
1.7
1.6
19.6
IL-4-PE
IFN-γ-FITC


Conclusions IV
•In vitro maturation and activation of DCs mirrors in vivo encounters with pathogens
•Bio-warfare vaccines anthrax and plague are poor immunogens as a consequence of poor DC stimulation
•Note *Nature* July 17 2003 Agrawal et al: anthrax LF severely impairs DC function; *J Exp Med* Oct 2002, Sing et al: Yersinia V antigen induces IL-10 from DCs
•Combinations of vaccines exhibit summative effects on DC activation and maturation

T helper cell responses to bio-warfare vaccines II



Conclusions V

- Recall responses to bio-warfare vaccines are detectable in Gulf War veterans 11-12 years after vaccination
- Preliminary results reflect mixed Th1/Th2/Tr1 immunity to anthrax, poorer immunity to plague

Summary

- Evidence of cellular immune activation in GW veterans, but analyses and relationship to vaccines difficult to evaluate, presumably due to time elapsed
- In vitro model of DC activation by vaccines provides a technology for assessing single vaccine effects, and for assessing multiple agents
- Multiple agents appear to have predictable, summative effects

Key collaborators

- Simon Wessely, Matthew Hotopf, King's College London
- Gareth Griffiths, DSTL, Porton Down, UK
- Martien Kapsenberg, Esther De Jong, U Amsterdam
- Funding: Medical Research Council, UK; US DoD

Presentation 10 - Al Marshall

Preliminary Assessment of DU Munitions Health Effects.

Al Marshall

National Security Studies Department
Sandia National Laboratories
Presented to RAC Gulf War Veteran's Illness
Washington DC
February 24, 2004

1

Who am I? Why am I studying DU?

- Sandia National Laboratories
  - DOE Lab (Nuclear Weapons, Energy)
  - National Security Studies department
- National Security Studies Dept.
  - Explore Terrorist Threats (DU?)
  - Objective studies of Sandia activities (U use)
  - DU issues of interest to department
- My Background
  - Physics, Nuclear Engineering
  - Physics orientation, not medical details

2

Objective: Explore DU exposure issues.

Examine:

- DU issues
- DU dispersal mechanisms
- Possible exposure mechanisms
- DU biokinetics
- Radiological health effects
- Heavy metal (chemical) health effects
- Report findings

3

Used Gulf War as DU case study.

DoD defined 3 exposure levels:

- Level I exposures: "Friendly fire" veterans
  - Accidentally targeted by US tanks
  - Highest exposures Highest exposures
  - Study completed

- Level II: Post-battle teams – No study planned
- Level III: Incidental Exposures – No study planned
- Civilian exposures – Study planned

Work in Progress

4

Preview of Preliminary Findings.

- Inhaled DU mass exceeds DoD estimates
- Fragment dose contribution significant
- DU radiological effect insignificant
- DU in Kidney high for max case, chemical heavy metal: consequences uncertain
- Other DU heavy metal effects possible, significance uncertain

5

Presentation Outline

1. DU Characteristics and Use
2. Intake and Biokinetics
3. Radiological Effects
4. Heavy Metal Effects
5. Summary and Conclusions

6

1. DU Characteristics and Use

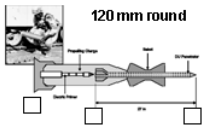
- Uranium:
  - Naturally occurring heavy metal, high density
  - Chemically toxic, slightly radioactive
  - Principal use: nuclear weapons, power reactors
- Natural isotopes:

Uranium Isotope	Z Protons	N Neutrons	A $Z+N$	half-life $T_{1/2}$ (yrs)	Support Chain Reaction?
U-234	92	142	234	2.5×10^5	No
U-235	92	143	235	7.0×10^8	Yes
U-238	92	147	238	4.5×10^9	No

7

DU use as armor penetrator.

- U enriched in U-235: reactors, nuclear weapons
- Leftover is DU, mostly U-238
- Ideal for armor penetration
  - Penetration depth ~density
  - Self sharpening
  - Inexpensive
  - Pyrophoric
    impact generates particles, explo



1991 Gulf War
- 900,000 DU rounds
- 315 tons DU

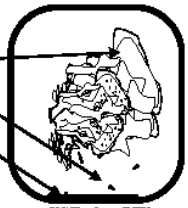
8

2. Intake and Biokinetics

Level I veteran exposure mechanisms.

- 1 or 2 120 mm rounds hit vehicle
- DU particulate, explosion

(1) Occupants inhale DU particles
(2) Occupants hit by DU fragments
(3) Occupants ingest DU powder



US Tank or BFV
Crew Compartment

9

(1) Look at inhalation exposure.

- Inhaled mass determines dose
- Estimated inhaled mass for nominal case

	This Study	DoD	Royal Society	Fetter and von Hippel
Mass (mg)	150	27	250	25
Approach	Both tank and BFV cases	Single tank test, air monitor data questioned	Crude modeling, Tank only	7 yr lag data taking Old model, old data
Reported validated by urine data?	Yes ?	Yes Old data	Yes Broadly consistent ?	Yes Old model, old data

10

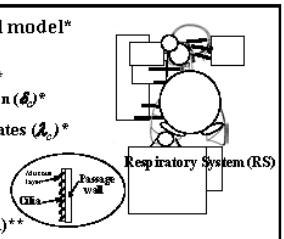
Estimated inhaled mass for maximum case.

	This Study	DoD	Royal Society	Fetter and von Hippel
Mass (mg)	**1850**	**237**	**5000**	**250**
Approach	Tank and BFV	Multiplier on nominal data -includes ingestion	Crude model Tank only	As for nominal
Reported consistent with urine data?	Yes	Not compared	Not compared	Yes Old data, model

11

Inhalation biokinetics: DU in RS.

- Recent international model*
  - –Particle deposition
    - •Particle size (d)**
    - •Deposition fraction (δ_D)*
  - –Particle transport rates (λ_{tr})*
    - •Ciliary action
    - •Lymph nodes
  - –Blood dissolution
    - •Rapid fraction (f_r)**
    - •Rapid, slow rates (λ_{rr})**

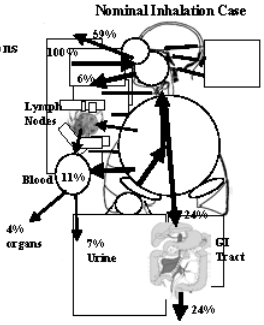


- Data from
  - *International Commission on Radiological Protection (ICRP)
  - **Impact particulate research

12

Basic distribution of inhaled DU.

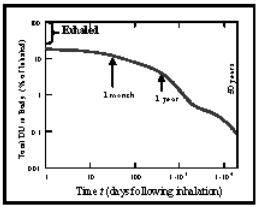
- **Basic equations solved**
  - Coupled differential equations
  - Compute time-dependent
    - Transport
    - Blood absorption
  - Each compartment
  - Rapid and slow blood
- **Equations couple to**
  - Other organ models
  - Urine elimination

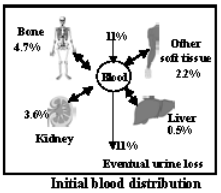


13

Blood distributes DU to organs

- Coupled to RS equations
- Total in body
  - ~60% exhaled, % remaining:
    - 10% at 2 months
    - 4% at 1 yr
    - < 0.01% at 50 yrs





Initial blood distribution to organs for DU

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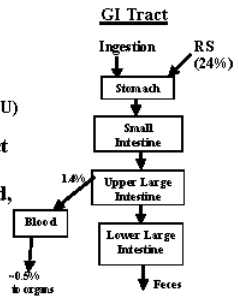
(2) Level I Ingested DU insignificant.

- Used ICRP model
- DU to GI tract from:
  - Ingestion (hand-to-mouth contact)
  - Respiratory System (RS inhaled DU)
- Passes rapidly through GI tract
- Small (1.4%) absorbed by blood, passed to organs
- Effect small for level I ignored here

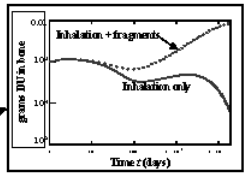


15

(3) Embedded fragments effect.

- Embedded fragment dissolution
  - Inferred dissolution rate from DU in urine (McDiarmid study)
  - Nominal case: Used average DU in urine
  - Maximum case: Used 2 x max DU in urine
  - Assume constant rate
  - Developed simple model
- Effect is significant

DU mass in bone nominal case



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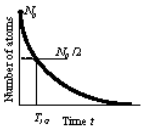
3. Radiological Effects

Background: Radioactive decay

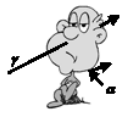
- Radioactive decay: emission of particles from nucleus
  - Disintegration (Decay)
    α, β, and γ
  - New element formed
- Activity: dis./sec
  - –Curies (Ci) = 3.7 x 10^{10} dis./sec
  - –Activity/g for U is very small
- Half life:
  - –Time for no. atoms to decay by 1/2
  - –Activity ~ $1/T_{1/2}$
  - –Long half-life = low activity



17

Types of radiation and exposures.

- Alpha (α) radiation
  - –External: no skin penetration, no health risk
  - –Internal: damage soft tissue, health risk
- Beta (β) radiation
  - –External: some penetration, skin burns
  - –Internal: damage soft tissue, health risk
- Gamma (γ) radiation
  - –External and internal:
    highly penetrating, health risk



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Dose determines health effects.

- Absorbed dose: Radiation energy absorbed
  - SI unit: Gray (Gy)
  - Older unit: rad (1 Gy = 100 rad)
- Dose equivalent: Dose x (W_r)
  - For radiation biological effect
  - SI unit: Sievert (Sv)
  - Older unit: (rem) 1 Sv = 100 rem
- Effective Dose: Dose x (W_r) x (W_t)
  - For organ sensitivity
  - W_t = 0.12 lung, bone marrow
  - W_t = 0.01 skin, bone surface
  - SI unit: Sievert (Sv)

Radiation	W_r
γ, β	1
α (internal)	20

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Level I Maximum individual dose.

- Lung dominates dose
- Max dose individual (Inhalation + fragments)
  - – 1st yr: = 190 mSv/yr
  - – 50 yr committed dose = 290 mSv
- Comparison
  - – Background: ~ 3 mSv/yr
  - – Worker limit: 50 mSv/yr
  - – Pack-a-day smoker: ~ 60 mSv/yr
  - – Worker 35 yr committed dose = 350 mSv/yr
- Fragment dose effect significant
  - – Organs 20 to 30 x inhaled dose
  - – Local effect?

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Level I Max Individual Radiological Effect.

- Linear No-threshold (LNT) model used
- Lifetime risk of radiation-induced fatal cancer:

  ~1 chance out of 70
- General population lifetime fatal cancer risk:

  ~1 chance out of 7
- Risk of radiation-induced birth defect:

  << 1 chance out of 10,000 (overestimate)
- General population risk of birth defect:

  ~1 chance out of 30

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Level I Collective Radiological Effects.

- Level I collective risk, assuming total for:
  - 60 individuals with fragments
  - 100 individuals inhalation-only
- Lifetime collective risks, radiation-induced fatal cancers:

  ~ 1 chance out of 5 any cancer in Any Level I Veteran
  (vs. ~24 fatal cancers for 160 individuals of general public)

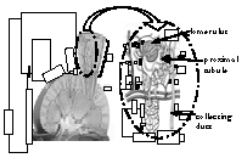
  ~ 1 chance out of 50 leukemia
  ~ 1 chance out of 100 bone tumor
  ~ 1 chance out of 3000 lymph cancer
  Any Level I Veteran

22

4. Heavy Metal Effects

Kidney is the target organ for DU.

- Heavy metal damage to kidney well established
  - Damage to proximal tubes
  - Depressed glomerluar function



Criterion (DOE*)	Kidney Burden μg DU / g kidney
No effect (chronic)	< 0.1
No effect* (acute)	< 1
Permitted*	< 3
LD/50*	55

2/3 kidney damage –without apparent symptoms

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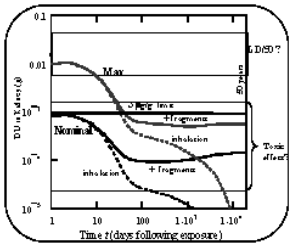
High DU mass in kidney predicted.

- Maximum case
  - No effects observed in vets
  - < 2 weeks: predict measurable effects
  - –> 2 g inhaled unlikely
  - –> 3 μg/g, < LD/50
- Kidney resilient, but
  - Total recovery unlikely
  - Acidosis Alkalosis?
    - Disorientation
    - Fatigue
    - Spasms
    - Nervousness



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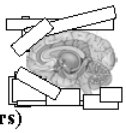
Comments.

- **Predicted risks are overestimates**
- **Lymph node W_t challenged**
  - Even if off by factor of 100, still small
- **Standard LNT challenged**
  - Too high? Too low?
  - Significant effect not likely
- **Other possible heavy metal effects**
  - Neurotoxic, hormonal, reproductive, cancer
  - Insufficient data to predict
  - Effect uncertain
- **U workers findings: (120,000 workers)**
  - Fatalities < avg. all cancers, kidney damage
  - Healthy worker effect?



25

5. Summary and Conclusions

- **Inhaled mass higher than DoD estimates**
- **Significant Fragment contribution to dose**
- **Radiological effect appears insignificant**
- **Max case Kidney DU heavy metal effect uncertain**
- **Other DU chemical effects possible**

All results are preliminary

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Presentation 11 – Terry Pellmar

Armed Forces Radiobiology Research Institute

Depleted Uranium Health Effects

Overview of AFRRI Research

A F R R I

Terry C. Pellmar, Ph.D.
Scientific Director

301-295-1211
pellmar@afrri.usuhs.mil
www.afrri.usuhs.mil

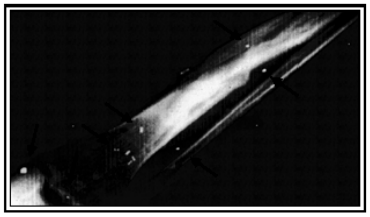
Health Effects of Depleted Uranium: AFRRI Research

Background

- *Gulf War involved first combat use of munitions made with depleted uranium (DU); only Coalition forces used DU; DU casualties resulted from friendly-fire incidents.*
- *Standard surgical guidelines dictate metal fragments be left in place if risk of surgery is greater than potential damage fragments may cause later.*
- *Given demonstrated effectiveness of DU munitions, U.S. can expect adversaries will use such weapons in future conflicts, resulting in much larger numbers of DU casualties.*
- *AFRRI research: are existing fragment removal guidelines appropriate for a metal with the unique chemical and radiological properties of DU?*

Health Effects of Depleted Uranium: AFRRI Research

DU shrapnel in lower leg



Health Effects of Depleted Uranium: AFRRI Research

EXPERIMENTAL APPROACH

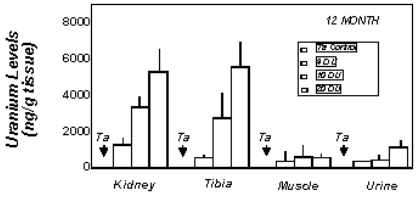
Rat model (Sprague-Dawley) with embedded DU pellets; in vitro studies with cultured cells (HOS)

- *Basic toxicological study: redistribution kinetics and evidence of toxicity; develop distribution model*
- *Assessment of carcinogenic potential*
- *Immunotoxicity*
- *Estimate risk and develop treatment strategies*

Health Effects of Depleted Uranium: AFRRI Research

DU Distribution and Toxicity...

Uranium Distribution in Rat Tissue after DU Pellet Implantation

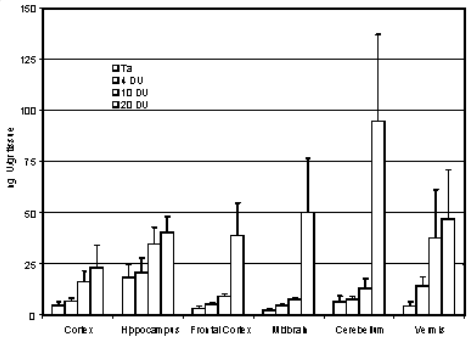


- *Uranium redistributes with time to various organs and tissues, especially bone and kidney. Uranium also distributes to brain, lymph nodes, and testes.*
- *No apparent changes in kidney or bone histology*

Pellmar et al., Toxicol. Sci. 49, 29-39 (1999)

Health Effects of Depleted Uranium: AFRRI Research

DU Distribution and Toxicity...



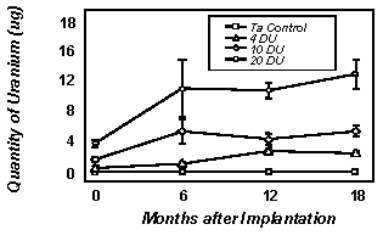
- *Non-homogeneous distribution of uranium in the brain*

Pellmar et al., Neurotoxicol. 20, 785-792 (1999)

Health Effects of Depleted Uranium: AFRRI Research

DU Distribution and Toxicity...

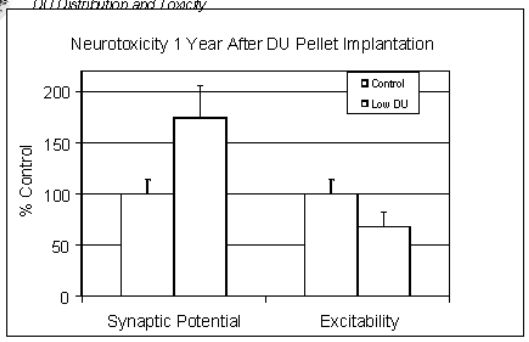
24-Hour Urine Levels



- *DU excretion rate stabilizes about 6 months after implantation*
- *No changes observed in urine glucose, protein, LDH and other markers of kidney function even with high levels of uranium in the kidney and urine*

Health Effects of Depleted Uranium: AFRRI Research

DU Distribution and Toxicity



- *DU altered electrophysiological activity in the hippocampus*
- *No gross behavioral changes were observed*

Pellmar et al., Neurotoxicol. 20, 785-792 (1999)

Health Effects of Depleted Uranium: AFRRI Research

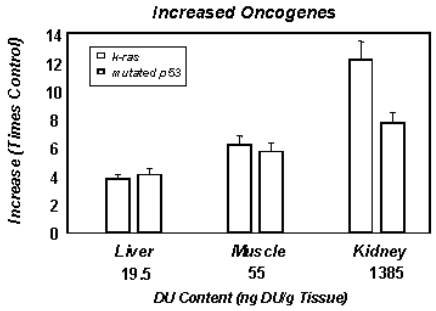
Transformation, Mutagenicity, Carcinogenicity

Principal Investigator: Alexandra Miller, Ph.D.

- Other heavy metals have been shown to be mutagenic and have the capacity to confer tumorigenic potential to exposed cells
- Determine whether exposure to embedded DU presents a long-term risk of cancer
- In Vivo: assess oncogene expression in DU-implanted animals; assess genetic instability in lymphocytes from implanted animals
- In Vitro: standard methodologies in cultured cells to assess both mutagenic and tumorigenic potential of exposure to DU

Health Effects of Depleted Uranium: AFRRI Research

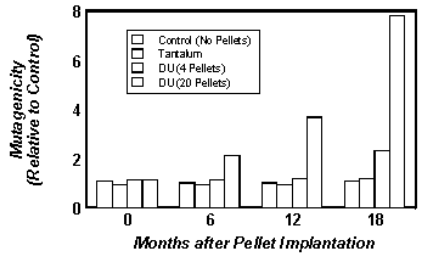
Transformation, Mutagenicity, Carcinogenicity...



- DU induces oncogenes known to be involved in carcinogenesis

Health Effects of Depleted Uranium: AFRRI Research

Transformation, Mutagenicity, Carcinogenicity...



- Urine from DU animals is mutagenic

Miller et al., Mutagenesis, 13, 643-648 (1998)

Health Effects of Depleted Uranium: AFRRI Research

Transformation, Mutagenicity, Carcinogenicity...

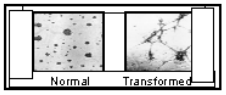
- DU and tungsten alloy metals induce genetic changes to extent similar to known carcinogens beryllium and nickel

	DU (Soluble)	DU (Insoluble)	WNiCo*	Be	Ni
Micronuclei Induction	↑	↑	↑	↑	↑
Sister Chromatid Exchange	↑	↑	↑	↑	↑
DNA Single-Strand Breaks	↑	↑	↑	(not done)	↑
Dicentric Formation	↑	↑	(not done)	(not done)	No Change

*WNiCo: reconstituted metal mixture of tungsten (W), nickel (Ni), and cobalt (Co) typical of tungsten military alloy

Health Effects of Depleted Uranium: AFRRI Research

Transformation, Mutagenicity, Carcinogenicity...



Normal and DU-Transformed HOS Cells

	Untreated	Tungsten	Tungsten/Nickel Cobalt	Nickel	Lead	Soluble DU	Insoluble DU	DU/Phenyl Acetate
Transformation Rate*	4.2	28.2	121.5	29.9	21.1	40.2	115.9	4.7
Tumorigenicity**	0 (0/82)	.33 (8/24)	.83 (10/12)	.29 (7/24)	.10 (2/20)	.44 (11/25)	.65 (13/20)	0 (0/12)

* Number of transformed cells per 500,000 surviving cells
** Number of tumors formed when 1 million transformed cells injected into immune compromised mice

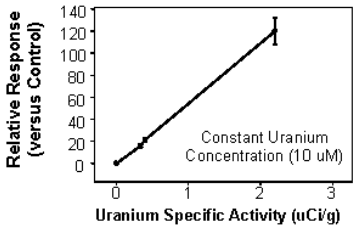
- DU transforms cells to a tumorigenic phenotype; cells form tumors in mice

Miller et al., Environ. Health Persp. 106, 465-471 (1998); Miller et al., Radiat. Res. (In Press)

Health Effects of Depleted Uranium: AFRRI Research

Transformation, Mutagenicity, Carcinogenicity...

Radiation vs. Chemical Toxicity of DU
Neoplastic Transformation Assay



- DU-induced transformation rate is influenced by radioactivity of DU, not just chemical toxicity

Miller et al.,Radiation Protection Dosimetry, submitted

Health Effects of Depleted Uranium: AFRRI Research

Immunotoxicity

Principal Investigators: David McClain, Ph.D. and John Kalinich, Ph.D.

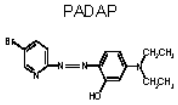
- Immune system is represented in a variety of tissues
- Other heavy metals have been shown to be immunotoxic
- AFRRI DU Distribution and Toxicity study determined there are alterations in several immune system parameters in DU-implanted rats

Health Effects of Depleted Uranium: AFRRI Research

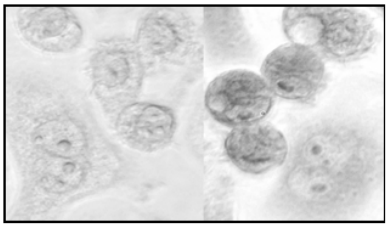
Immunotoxicity

PADAP Staining of DU-treated J774 Cells

PADAP



2-(5-Bromo-2-pyridylazo)-5-diethylaminophenol

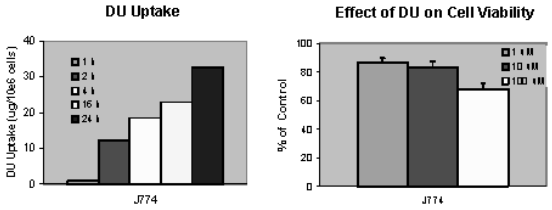


Without DU With DU

- Uranium-specific dye labels cells that take up the metal

Health Effects of Depleted Uranium: AFRRI Research

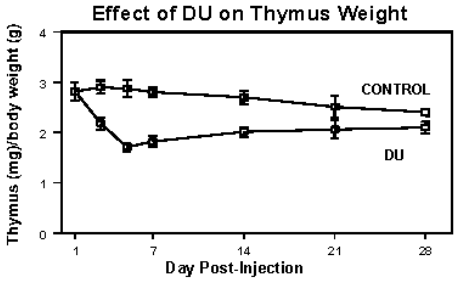
Immunotoxicity



- *Soluble DU is taken up and concentrated by cultured macrophages, affecting their metabolic viability, and eventually leading to their death*

Health Effects of Depleted Uranium: AFRRI Research

Immunotoxicity



- *A variety of hematological parameters (immune organ weight and cellularity, lymphocyte number) are altered in mice injected with soluble DU compounds*
- *Cytotoxic T-cells are significantly elevated in blood from DU-exposed animals*

Health Effects of Depleted Uranium: AFRRI Research

Future Directions

- *Expand DU carcinogenicity study (in vitro and in vivo); include other heavy metals of military interest*
- *Expand in vivo immunotoxicity assessment*
- *Evaluate transgenerational effects of in vivo exposure to DU in both male and female rodents*

Health Effects of Depleted Uranium: AFRRI Research

AFRRI DU Research Team

David McClain PhD
John Kalinich PhD
Christy Emond
SSgt Michael Stewart
Kia Brooks
Alexandra Miller PhD
LT Blaise LeBlanc PhD
Tom Dalton
Vilmar Villa
LT Shelly Hakspiel

Presentation 12 – Johnnye Lewis

Inhalation of Uranium Oxides to Mimic Gulf War Exposures:

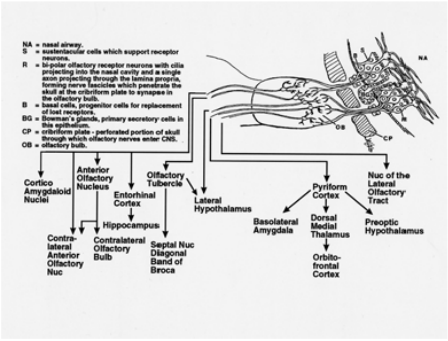
Deposition and toxicity in brain, lung, and kidney

Johnnye Lewis, Ph.D., DABT
Director, Community Environmental Health Program, University of New Mexico Health Sciences Center

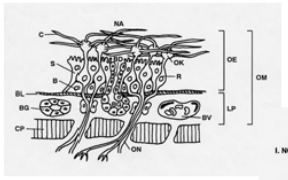
Co-Investigators:
Graham Bench, Ph.D., CAMS, Lawrence Livermore National Laboratory
Fletcher Hahn, DVM, Ph.D., DACVP, Lovelace Respiratory Research Institute
Jenny Karlsson, Ph.D., Community Environmental Health Program, UNM HSC
Ed Barr, MSEE, Lovelace Respiratory Research Institute

Background of Team

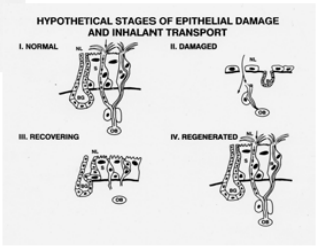
Nose-Brain Barrier



Epithelial structure, damage, & uptake



Normal Epithelium



Perspective on Gulf War Exposures and Disease

What were unique characteristics of Gulf War exposures?

- **Multiple toxicant exposures (+neurotoxicants)**
- **Inhalation – major route of exposure**
- **Potential for sensitization**
  - Many irritants – dusts, smoke, petroleum combustion products
- **Acute and chronic exposures both likely**

DU as a contributor

- **Potential for exposures to DU aerosols**
  - Tank-Impact – High concentration, acute (15 min) exposure
  - March-Through – Low concentration – single day
  - Clean-up – Low concentration – up to 30 day
  - Maintenance – Very low concentration – longer duration
- **Aerosols resulted from impact, combustion, resuspension**
  - Estimates of exposure inconsistent
    - Varied from 300 micrograms to >25 grams
    - Estimates of solubility and respirability varied
  - Respirable fraction could move suspended for hours
- **Other heavy metals neurotoxic and neuroimmunotoxic**

Assembly of multidisciplinary team

...collaborative history

- Inhalation toxicology of metals and neurotox – *J. Lewis, DABT & J. Karlsson (UNM)*
  - **Olfactory uptake of inhaled metals to CNS – *Nose-Brain Barrier***
  - **Neurochem/anatomy of neurodegenerative disease**
- Generation of complex respirable aerosols – *E. Barr (LRRI)*
  - **>20 years of aerosol generation history – metals, solvents, rads**
  - **Studies of factors influencing respiratory tract deposition**
- Quantitative localization of metals – *G. Bench (LLNL)*
  - **Micro-PIXE analysis – high resolution, low detection limit, single-scan – multiple metal analysis; History with biologic tissues**
- Pathology of kidney and lung – *F. Hahn, DAVP (LRRI)*
  - **Historical work in DU shrapnel implantation**
  - **Pathology of inhaled metals and radionuclides**

Hypotheses

1. *Inhalation of uranium aerosols during the Gulf War from combustion of DU-containing weapons resulted in CNS deposition and subsequent neurodegeneration in a subset of those exposed*
2. *Transient conditions which compromise the olfactory epithelium will enhance the entry of uranium and the subsequent development of neurodegeneration*
3. *Markers of neurodegeneration will be correlated with the concentration and pattern of deposition of uranium within the CNS following inhalation exposure*

Scope:

- Expose rats by inhalation to aerosols of uranium varying in solubility, to tantalum oxide, or to air
  - *Tank-Impact Scenario:*
    - **Acute (15 min) – high-level concentrations (500 mg/m^3)**
  - *March-Through Scenario:*
    - **Short duration – moderate concentration (1 mg/m^3 - 6 hrs)**
  - *Clean-Up Scenario:*
    - **Longer duration – moderate concentration (1 mg/m^3/ 6 hrs/ 30 days)**
  - *Maintenance Scenario:*
    - **Long-term – low concentration (0.01 mg/m^3/ 6 hrs/ 30 days)**

Scope (cont'd):

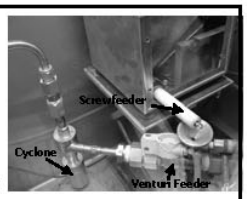
- Expose with and without concommitant respiratory tract inflammation
- Localize U & assess pathology in CNS, lung, kidney
- Assess time course of response through serial sacrifices at 0, 30, 180, and 360 days post-exposure

Importance of team experience

- Relevant exposure methods
  - Magnitude of effect, target organ, and overall toxicity dependant on route and pattern of exposure
- Physiologic exposures
  - Physiological defense mechanisms can be swamped by excessive exposure concentrations
- Intra regional localization *in situ*
  - Non-homogeneous deposition can be diluted by inclusion of non-affected tissues
- History with sensitization protocols

Glove Box Enclosure System

Aerosol Generation System



Exposure Chamber Pass Box

96-Port Nose-Only Exposure Chamber

EXPOSURE

Ed Barr, MSEE
Lovelace Respiratory Research Institute

Uranium atmosphere

Tank-impact scenario

Compound	Conc [mg/m³]	Size Distribution MMAD, μm	GSD
Ta_2O_5	548	2.1	1.9
UO_2	329	1.6	1.7
DUOx	609	2.0	1.4
UO_2	572	2.4	1.4
$UO_2 + UO_2$	305	2.0	1.5

Air

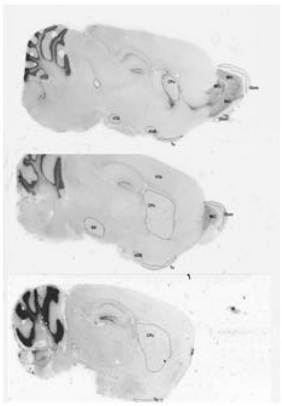
To test sensitization, endotoxin used to induce nasal inflammation

$UO_2 + UO_2$ + endotoxin
DUOx + endotoxin
Air + endotoxin

U analysis via Nuclear Microscopy

Graham Bench

**Center for Accelerator Mass Spectrometry,
Lawrence Livermore National Laboratory,
Livermore, CA 94550**



Brain Tissue Prep

Dissection:

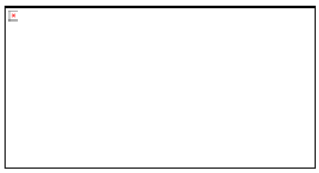
Three sagittal planes identified to encompass brain regions of interest

PIXE at early time points – frozen tissue only

Longer survival, fixation

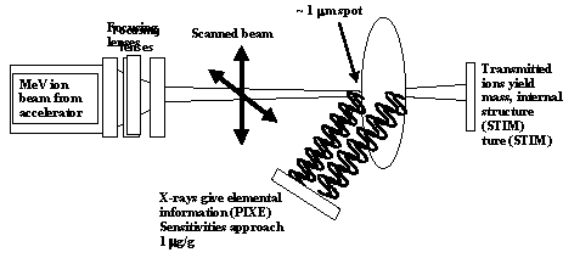
Preparation of tissue sections for PIXE analysis

- Bilateral sagittal 10 micron cryosections mounted on nylon foils and freeze-dried
- Adjacent sections mounted onto glass slides for immunohistochemical analysis or cytological staining.
- Adjacent slide-mounted sections stained cytologically, photographed and regions of interest identified



- Marked images sent as portable document files (pdf) along with the freeze dried sections to LLNL for analysis.

Nuclear microscopy: spatially resolved elemental and mass quantitation



PIXE - Proton Induced X-ray Emission

STIM - Scanning Transmission Ion Microscopy

Element profiles within identified regions measured with PIXE

- Structures on adjacent stained sections identified on serial freeze dried section with a x 40 optical microscope
- PIXE utilized 3 MeV protons to produce x-ray spectra.
- STIM measured the residual energy of the proton beam after it had passed through the sample.
- Beam spot sizes varying between 0.2 x 0.2 and 0.5 x 0.5 mm were used to irradiate brain regions for 15 microcoulombs of charge.
- X-ray spectra were analyzed and the incoming and outgoing energies of the proton beam as it traversed the sample were used to convert x-ray yields to concentration in units of mg/kg using the the PIXEF analysis package (PIXEF: *The Livermore PIXE Spectrum Analysis Package. A.J. Antolak and G. Bench. Nucl. Instr. and Meth.* ***B90****, (1994), 596-601).*

Deposition at 4 hr post-exposure

Tank-Impact Scenario

Kidney metal uptake

At 4 hr post "tank-impact" exposures, only UO_3 animals showed detectable U in kidneys

Max concentration expected at 7 days post exposure

Pooled tissue to increase sensitivity - UO3 early deaths

Day 6 death (n=5)	34.2 ± 2.1 mg/kg U dry weight
Day 7 death (n=3)	34.6 ± 1.7 mg/kg U dry weight
Day 8 death (n=3)	24.6 ± 1.7 mg/kg U dry weight
Day 10 and 13 death (n=2)	23.4 ± 1.3 mg/kg U dry weight.

Uranium uptake in brains

4 hr post-15 min high dose exposure

Characterization of MDLs across Brain Regions Analyzed

Structure	MDL	SE	95% CI Lower	95% CI Upper
CPu	2.53	0.05	2.43	2.63
Glomeruli	2.60	0.03	2.53	2.66
Mitral	2.62	0.03	2.56	2.69
SN	2.55	0.05	2.45	2.65
Sp	2.67	0.05	2.58	2.76
Tu	2.43	0.05	2.33	2.53
Overall	2.58	0.02	2.54	2.62

No detectable uptake – regardless of form

Pathology at 4 hr post-exposure

Tank-Impact Scenario

(Moribund sacs & deaths at <14 d included)

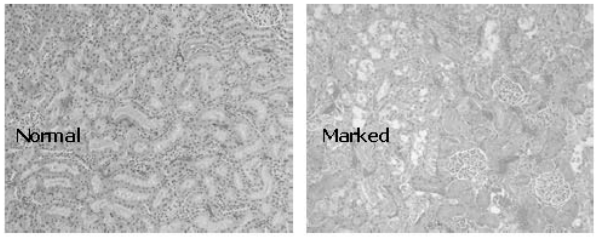
Early Deaths and Moribund Sacrifice of Rats After Inhalation Exposure to UO_3

Days Post Exposure	Number[a] M/F	Histologic Score[b] Kidney Tubular Necrosis M	F	Lung Uremic Pneumonia M	F
2	1/0	0	-	0	-
4	0/1	-	4	-	2
6	0/5	-	3.8	-	3.4
7	0/3	-	3.3	-	4
8	1/2	4	4	3	4
10	0/1	-	3	-	3
13	1/0	3	-	3	-
	3/12				

[a] Initial number at risk: 34 M; 34 F
[b] 2 = Mild; 3 = Moderate; 4 = Marked

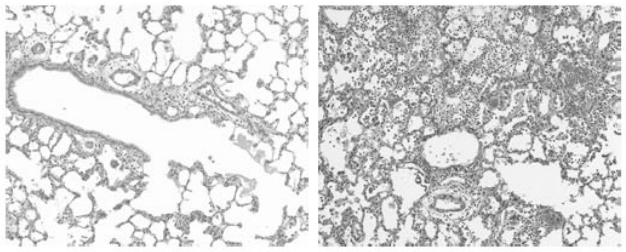
Renal Tubular Necrosis

More soluble UO_3 resulted in renal tubular necrosis and uremia



Uremic Pneumonia

Mild vs. Marked, Fatal



- **Uremic pneumonia was the immediate cause of death**
- **Females had a higher death rate**

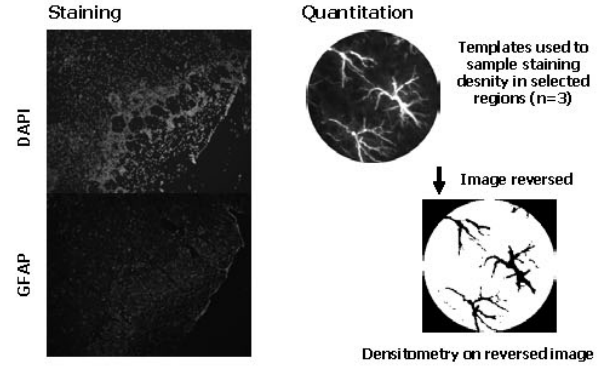
Lung Pathology – 4 hr post *Tank-Impact*

Exposure Atmosphere	Number M/F	Lung Histologic Score (Ave)[a] Alveolar Macrophage Particles M/F	Alveolar Macrophage Hyperplasia M/F
Air	4/4	0 / 0	0 / .25
UO_2	3/3	1.3 / .66	1 / .66
UO_3	3/3	0 / 1	0 / .66
UO_2 +UO_3	3/3	0 / .66	1 / 1.3
TaO_2	3/3	.33 / 1	0 / .66
DUO	3/3	.33 / .66	.33 / .66
Endotoxin	3/3	0 / 0	1 / .66
UO_2 +UO_3 + Endotoxin	3/3	.66 / .33	.33 / 1.3
DUO + Endotoxin	3/3	0 / 1	1 / 1

[a] 1=Minimal; 2 = Mild

- Few particles found in sections, no concentration at broncho-alveolar junction
- Nearly all particles in alveolar macrophages
- Number of alveolar macrophages only minimally increased

GFAP methods



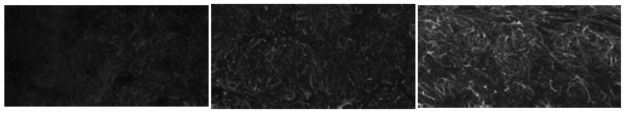
Brain inflammation – GFAP data

Tank-impact scenario

- Solubility related increase in GFAP
- Endotoxin increases GFAP response in all exposures

GFAP 4 hours post 15 min high dose exposure

Tank-impact Scenario

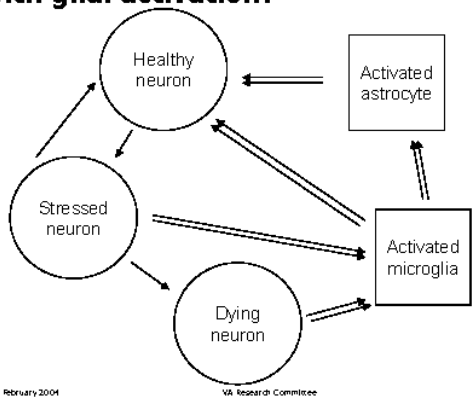


Control DUOx UO3

What is the significance of glial activation?

- **Are glia synthesizing & releasing cytokines?**
  - Immunohistochemistry cytokine patterns
  - Protective or degenerative response?
  - Proinflammatory
    - IL-1, IL-6, TNFα or
  - Antiinflammatory
    - IL-10, TGFβ1
- **What are microglia doing?**
  - OX-42, daintain (AIF-1)

What is happening to neurons in regions with glial activation?



Low dose, longer duration U exposures

March-Through Scenario
Clean-up Scenario

Exposure Characterization

- **Nose-only inhalation, rats, male and female**
- **1 or 30 day exposure (6 h/day. 5 days/week)**
- **UO_2:UO_3, 1:1 mixture**
- **Target conc 1 mg/m3**
  - Actual conc 1.02 +/- 0.12 mg/m3
  - Size 1.66 +/- 0.01 micron
  - sigma-g 1.55 +/- 0.11
- **Sacrifices 0, 30, 180, 360 days post-exposure**

Results -- 4 hr post-exposure

- ***Clean-Up Scenario*** (30 d x 6 hr – 1 mg/m³ UO_3+UO_2)
  - no endotoxin – no uptake observed
- ***March-Through Scenario*** (1 d x 6 hr – 1 mg/m³ UO_3+UO_2)
  - no endotoxin – no uptake observed
- ***March-Through Scenario with nasal inflammation***
  - *2 of 6 animals show uptake in glomerulus, 1 also in deeper mitral cell layer*

Conclusions

- **Very Short/High Dose – *Tank-Impact* scenario**
  - no detectable CNS uptake regardless of solubility
  - Solubility-related neuroinflammation
  - Most soluble forms result in extensive renal deposition and renal toxicity
  - Females more sensitive to renal toxicity
- **Short-term/ Moderate Dose – *March-Through* Scenario**
  - Nasal inflammation increases the probability of CNS deposition and transport with low dose inhalation for 6 hr durations
- **Longer-duration/ Moderate Dose – *Clean-Up* Scenario**
  - No uptake observable in animals without inflammation

In progress

- **Characterization of nasal inflammation**
- **30 day exposure with inflammation**
- **Characterizing longer survival times**
- **Continued analysis of neurotoxicity at longer survival times**
- **Additional exposures at lower doses (*Maintenance* Scenario)**

Acknowledgements

- This research was funded by Department of Defense Award
  - DAMD-17-01-1-0794
- We would like to acknowledge the technical support of
  - Wendy Barrington (UNM)
  - Rhonda Garlick (UNM & LRRI)
  - Antonia Abeyta (UNM)
  - Dee Esparza (LRRI)

Presentation 13 - Melissa McDiarmid

HEALTH EFFECTS OF DEPLETED URANIUM IN EXPOSED GULF WAR VETERANS – A TEN-YEAR FOLLOW-UP

Melissa A. McDiarmid, M.D., M.P.H.
VA Maryland Health Care System
Baltimore Division

Background

- Friendly Fire incidents - inhalation exposure/wound contamination and embedded shrapnel
- Finding - relation between shrapnel status and elevated urinary uranium first observed in 1994 visit, confirmed in all 4 subsequent visits

Purpose of DU Surveillance Program

- Determine health effects, if any, in exposed population
- Develop methods to measure uranium exposure in novel exposure mode (embedded shrapnel)
- Examine surgical management of shrapnel

Summary of Surveillance Visits

Year	Cases	Non-exposed	Total
1993-4	33		33
1997	29	38	67
1999	21+29 new		50
2001	31+8 new (17 original cases)		39
2003	32		32

A total of 70 individuals involved in friendly fire incidents have been evaluated at Baltimore.

Surveillance Protocol
Detailed Questionnaire

- Medical History
- Social History
- Family History
- Occupational/Exposure History
- Reproductive History
- Partner's History

Surveillance Protocol
Laboratory Studies

- CBC
- Blood Chemistries
- Urinalysis
- Neuroendocrine markers (FSH, LH, Prolactin, Testosterone)
- Urinary Uranium
- Immunologic markers
- Other Uranium Measures

Surveillance Protocol
Special Studies

- Semen analysis
- Chromosomal aberrations
- Sister chromatid exchange

Surveillance Protocol
Additional Surveillance Components

- Physical examination
- Neurocognitive test battery
- Whole body radiation counting
- Risk Communication/Focus Group

Demographic Characteristics for 2001 Cohort

	N	%
RACE		
African-American	12	31
Caucasian	22	56
Hispanic	4	10
Other	1	3
EDUCATION		
0-8 years	1	3
9-12 years	9	23
Some college	22	56
College degree	4	10
Post college	3	8
MARITAL STATUS[a]		
Never married	3	8
Married	31	79
Divorced	4	10
Unknown	1	3
AGE[b]	35.1 ± 0.76	

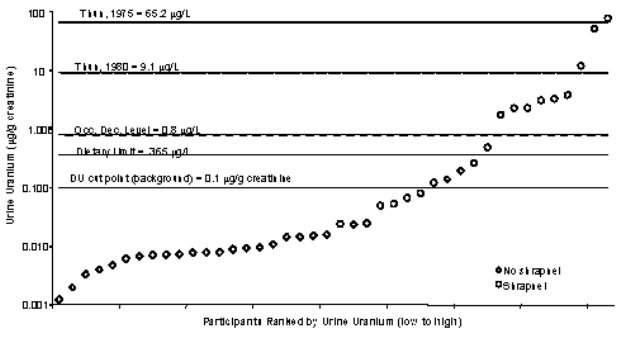
[a] At time of 2001 evaluation
[b] Mean age at time of 2001 evaluation (±SE, standard error of the mean)

Active Medical Problems Summary (2001)

	Low Uranium Group[a]		High Uranium Group[b]		Mann-Whitney Test(p)
	n	%	n	%	
Participants with active problems[c]	26	100	12	92.3	0.15
Injuries[d]	7	26.9	13	100	0.00
Musculoskeletal	17	65.4	7	53.8	0.49
Cardiovascular	10	38.5	2	15.4	0.14
Psychiatric	4	15.4	3	32.1	0.56
Nervous system	5	19.2	3	23.1	0.78
Other[e]	18	69.2	10	76.9	0.61

[a] < 0.10 µg/g creatinine (n=26)
[b] ≥ 0.10 µg/g creatinine (n=13)
[c] Individuals might have more than one problem
[d] Injuries sustained during Gulf War
[e] Gastrointestinal, skin, respiratory, genitourinary

Urine Uranium (2001) N=39



Hematologic Parameters Summary (2001)

Laboratory test (normal range)	Low Uranium Group[a] (mean ± SE)	High Uranium Group[b] (mean ± SE)	Mann - Whitney Test (p)
White Blood Cells (4.8-10.8 K/cm^3)	6.53 ± 0.41	5.85 ± 0.45	0.36
Hematocrit (42-52%)	44.60 ± 0.43	42.59 ± 0.80	0.03
Hemoglobin (14-18 g/dL)	15.40 ± 0.15	14.79 ± 0.32	0.07
Platelets (140-440 K/cm^3)	254.54 ± 13.82	234.08 ± 13.73	0.21
Lymphocytes (%) (15-45%)	36.87 ± 1.99	36.07 ± 1.81	0.80
Neutrophils (%) (40-75%)	50.95 ± 2.09	51.83 ± 1.97	0.74
Basophils (%) (0-2%)	0.78 ± 0.10	0.65 ± 0.07	0.54
Eosinophils (%) (0-4%)	3.60 ± 0.35	3.51 ± 0.44	0.85
Monocytes (%) (2-12%)	7.79 ±0.37	7.94 ± 0.48	0.99

[a] < 0.10 µg/g creatinine (n=26)
[b] ≥ 0.10 µg/g creatinine (n=13)

Renal Function Parameters (2001)

Laboratory test (normal range)	Low Uranium Group[a] (mean ± SE)	High Uranium Group[b] (mean ± SE)	Mann - Whitney Test (p)
Serum creatinine (0.5-1.1 mg/dL)	0.95 ± 0.03	0.85 ± 0.03	0.03
Serum uric acid (3.4-7 mg/dL)	5.94 ± 0.23	5.85 ± 0.51	0.45
Serum calcium (8.4-10.2 mg/dl)	9.17 ± 0.006	9.27 ± 0.137	0.67
Serum PO4 (2.7-4.5 mg.dl)	3.82 ± 0.101	3.82 ± 0.148	0.63
Urine calcium (100-300 mg/24 hr)	183.50 ± 23.8	214.50 ± 26.3	0.35
Urine PO4 (0.4-1.3 g/24 hr)	1.03 ± 0.008	1.15 ± 0.107	0.40

[a] < 0.10 µg/g creatinine (n=26)
[b] ≥ 0.10 µg/g creatinine (n=13)

Renal Function Parameters (2001) cont.

Laboratory test (normal range)	Low Uranium Group[a] (mean ± SE)	High Uranium Group[b] (mean ± SE)	Mann - Whitney Test (p)
Urine beta-2 microglobulin (0-300 µg/g creatinine)[c]	38.53 ± 6.71	36.42 ± 7.46	0.78
Urine retinol binding protein (3-610 µg/g creatinine)	46.13 ± 3.46	65.68 ± 11.11	0.06
Urine creatinine (1.3 -2.6 g/24 hr)	1.99 ± 0.11	2.14 ± 0.10	0.29
Urine total protein (0-92.8 mg/g creatinine)	54.63 ± 4.94	78.69 ± 10.52	0.01

[a] < 0.10 µg/g creatinine (n=26)
[b] ≥ 0.10 µg/g creatinine (n=13)
[c] n = 16 for low uranium group and n = 9 for high uranium group

Neurocognitive Impairment Measures (2001)

Laboratory test	Low Uranium Group[a] (mean ± SE)	High Uranium Group[b] (mean ± SE)	Mann - Whitney Test (p)
A-Ilac: Indexes Accuracy	0.16 ± 0.04	0.27 ± 0.07	0.138
A-Iirt: Indexes Speed	0.14 ± 0.03	0.14 ± 0.06	0.886
A-Iitp: Indexes Accuracy per Minute	0.12 ± 0.02	0.17 ± 0.06	0.717
NP3 Index	0.05 ± 0.01	0.09 ± 0.04	0.812

[a] < 0.10 µg/g creatinine (n=26)
[b] ≥ 0.10 µg/g creatinine (n=13)

Neuroendocrine and Thyroid Hormone Parameters (2001)

Laboratory test (normal range)	Low Uranium Group[a] (mean ± SE)	High Uranium Group[b] (mean ± SE)	Mann - Whitney Test (p)
Prolactin (2.1 - 17.7 ng/mL)	18.84 ± 1.60	14.70 ± 2.76	0.06
FSH[c] (.9-15 IU/ml)	4.39 ± 0.50	4.51 ± 0.74	0.95
LH[c] (1.5-9.3 mIU/ml)	5.09 ± 0.51	5.13 ± 1.04	0.48
Testosterone (3-10 ng/ml)	5.64 ± 0.49	4.77 ±0.47	0.28
TSH[c] (0.49-4.67 µIU/ml))	1.99 ±0.24	2.28 ± 0.50	0.89
Free thyroxine (0.71-1.85 ng/dL)	1.66 ± 0.35	1.08 ± 0.07	0.02

[a] < 0.10 µg/g creatinine (n=26)
[b] ≥ 0.10 µg/g creatinine (n=13)
[c] FSH, follicle - stimulating hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone

Semen Characteristics (2001)

Clinical parameters (normal range)	Low Uranium Group[a] (mean ± SE)	High Uranium Group[b] (mean ± SE)	Mann - Whitney Test (p)
Days Abstinence (2 – 5 days)	4.8 ± 1.7	4.2 ± 0.9	0.820
Semen Volume (2-5 ml)	2.6 ± 0.4	3.5 ± 0.6	0.167
Sperm Concentration(> 20 million/mL)	102.8 ± 28.6	219.1 ± 70.5	0.126
Total Sperm Count (>40 million)	241.6 ± 66.4	708.6 ± 215.1	0.061
Percent Motile Sperm (>50%)	57.6 ± 4.9	60.5 ± 6.3	0.639
Percent Progressive Sperm [WHO[c] Class A and B] (>50%)	27.3 ± 3.2	25.7 ± 3.7	0.766
Total Progressive Sperm [WHO Class A and B] (>20million)	79.9 ± 22.6	206.8 ± 58.3	0.126
Percent Rapid Progressive Sperm [WHO Class A] (>25%)	17.6 ± 2.7	16.3 ± 2.5	0.586
Total Rapid Progressive Sperm [WHO Class A] (>10 million)	54.9 ± 16.2	134.8 ± 40.5	0.152

[a] < 0.10 µg/g creatinine (n=16)
[b] ≥ 0.10 µg/g creatinine (n=11)
[c] WHO, World Health Organization

Genotoxicity Parameters (2001)

Laboratory test	Low Uranium Group[a] (mean ± SE(n))	High Uranium Group[b] (mean ± SE(n))	Mann - Whitney Test (p)
Mean aberrations/cell	0.003 ± 0.001 (26)	0.01 ± 0.004 (13)	0.027
Mean SCE[c] untreated	5.07 ± 0.32 (25)	4.39 ± 0.37 (13)	0.199
Mean SCE w/Bleomycin 2 µg/ml	5.42 ± 0.32 (23)	5.95 ± 0.71 (11)	0.663
Mean SCE w/Bleomycin 4 µg/ml	6.31 ± 0.60 (20)	5.30 ± 0.42 (11)	0.197
HPRT MF[d]	10.97 ± 0.97 (26)	19.84 ± 4.89 (13)	0.105

[a] < 0.10 µg/g creatinine
[b] ≥ 0.10 µg/g creatinine
[c] SCE, sister chromatid exchange
[d] HPRT MF, hypoxanthine phosphoribosyl transferase mutation frequency

Radiation Dose Estimate from Whole Body Counting

- Nine veterans with whole body measurements above background
- Radiation dose estimates calculated using ICRP 30 Biokinetic model for U
  - 0.01 to 0.11 rem/year
  - 0.61 to 5.33 rem/50 years
- Public dose limit: 0.1 rem/year
- Occupational limit: 5 rem/year

Immunologic Markers (2001)

Laboratory test (normal range)	Low Uranium Group[a] (mean ± SE)	High Uranium Group[b] (mean ± SE)	Mann - Whitney Test (p)
IGG (690-1400 mg/dL)	1239.04 ± 68.13	1243.46 ± 75.46	0.82
IGA (88-410 mg dL)	199.00 ± 16.33	198.69 ± 24.66	0.95
IGM (34-210 mg/dL)	110.19 ± 12.03	96.85 ± 10.03	0.79
Complement C3 (75-140 mg/dL)[c]	126.32 ± 4.66	123.85 ± 8.20	0.56
Complement C4 (10-34 mg/dL)[c]	24.52 ± 1.47	26.62 ± 2.17	0.89
C-reactive protein	0.91 ± 0.04	0.95 ± 0.03	0.76

[a] < 0.10 µg/g creatinine (n=26)
[b] ≥ 0.10 µg/g creatinine (n=13)
[c] N for low uranium group, 25

Depleted Uranium Follow-Up Program Collaborators

- Melissa McDiarmid, MD, MPH
- Katherine Squibb, PhD
- Susan Engelhardt, RN, MN
- Marc Oliver, RN, MPH
- Patricia Gucer, PhD
- Craig Thorne, MD, MPH
- Robert Kane, PhD
- Michael Kabat, PhD
- John Ejnik, PhD
- Robert Ououa, PhD
- Barbara Curbow, PhD
- Larry Anderson, PhD
- Dennis Hoover, PhD
- Richard Albertini, MD
- Bruce Kaup, MD
- Lawrence Brown, MD
- David Jacobson-Kram, PhD

Presentation 14 – Lea Steele

Research Advisory Committee on Gulf War Veterans' Illnesses
February 23-24, 2004

Overview of Research on Infectious Diseases in Gulf War Veterans

Lea Steele, Ph.D.

RAC-GWVI
Research on Infectious Diseases in Gulf War Veterans

- Overview: Assessment of Infectious Diseases in Gulf Vets
- Leishmaniasis
- Mycoplasma

RAC-GWVI
Research on Infectious Diseases in Gulf War Veterans

- Infectious Disease Concerns during the Gulf War
  - Routine, familiar infections (URI, GI, skin, etc)
  - Regional organisms to which allied troops might not have immunity *(esp. shigellosis, malaria, sand fly fever, cutaneous Leishmaniasis)*
  - Food, water contamination
  - Vaccine contamination?
  - Use of biological weapons?

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Research on Infectious Diseases in Gulf War Veterans

Assessments of Infectious Diseases in CDC Study of Gulf veterans in PA Air National Guard (Fukuda et al, JAMA1998 280:981-8)

- 99 multisymptom illness case Gulf veterans vs. 59 controls
- Evaluated: Stool specimens for multiple organisms
  Serologic (antibody) testing for multiple organisms
- Stool specimens: no salmonella, shigella, campylobacter, yersinia, e.coli, microsporidia, cryptosporidium, cyclospora
- Serology: no antibodies to West Nile, Toscana, Karimbad, Isfahan, shistosomiasis species

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Research on Infectious Diseases in Gulf War Veterans

	Stool Specimen Testing
Blastocystis hominis	7% cases, 12% controls
Giardia	1% of mild cases, 2% of controls
Enteroviruses	9% of mild cases, 10% of controls
	Serologic Testing
Yellow fever	83% positive (due to vaccine), no difference by case status
Botulinum toxin	6% positive, no difference by case status
Anthrax PA	9% positive, no difference by case status
Leishmania	5% positive; no difference by case status
Toxoplasma gondii	19% positive, no difference by case status
Dengue fever	10% positive, no difference by case status
Sand fly fever	9% cases, 2% controls

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Research on Infectious Diseases in Gulf War Veterans

Human Herpesviruses (Wallace et al, Clin Diag Lab Imm 1999 6:216-223)

- 46 Gulf veterans who met criteria for chronic fatigue syndrome vs. 32 in good health
- Evaluated: Antibody titers to HHV6 and EBV
  PCR for HHV6, HHV7, EBV, CMV in periph mononuclear cells
- Found no differences by serology or PCR between sick and healthy veterans
- Gulf veterans, overall, had lower prevalence of herpes virus DNA than civilians

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Research on Infectious Diseases in Gulf War Veterans

Dr. Edward Hyman: "Antibacterial Treatment Method Based on Excretion of Dead and Dying Spherical Bacteria"

- Study done 1997-1999; info from DOD summary report
- 36 symptomatic veterans with coccal bacteria in urine randomized to treatment and placebo groups
- Treated with IV antibiotics according to level of excreted cocci
- Project report indicates "Results show a highly significant benefit in relieving fatigue and headache and in improving 'quality of life'. The results in improving pain approached but did not reach a p=0.05."

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Research on Infectious Diseases in Gulf War Veterans

Leishmania

- Several species endemic to the Middle East, had been a problem for foreign troops in World War II
- *L. tropica* usually causes cutaneous infection, but was associated with viscerotropic leishmaniasis in at least 12 Gulf veterans
- Systemic infection can be associated with chronic fatigue, abdominal pain and diarrhea, other symptoms
- Actual number of cases unknown: no sensitive screening test available
- Preliminary prevalence study done by Portland VAMC

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Prevalence of *Leishmania tropica* in a random sample of 200 Gulf War veterans (D Bourdette, M Riscoe, R Houghton, S Reed et al)

- First 200 subjects in population-based study tested for reactivity to *L.tropica* recombinant protein using an ELISA test.
- Samples considered positive if values > 3 SDs above the mean value in a population of healthy, nonveteran controls
- Positive serology found in 18 (9%) veterans; none had evidence of clinically active leishmaniasis

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Leishmania tropica and GWI case/control status

- 110 Gulf veteran GWI cases; 57 controls
  (cases: 1 or more of musculoskeletal pain, cognitive problems, gastrointestinal problems, skin lesions, fatigue)
- Antibody positive: 10% cases, 4% controls (exact p value = 0.149)
- Remaining subjects not assessed, findings not followed-up
- Sensitivity/specificity of test not known

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Research on Infectious Diseases in Gulf War Veterans

Mycoplasma infection

- *Mycoplasma* are small bacteria that lack a cell wall, but are capable of independent self-replication.
- *Mycoplasma* species are associated with human diseases affecting a variety of organ systems (e.g., *m. pneumoniae*, *m. genitalium*, *m. hominis*). They can be present without causing illness or can cause chronic infections, and can be particularly aggressive in immunocompromised patients
- *Mycoplasma fermentans* has been isolated from human saliva, urogenital tract, respiratory tract, bone, joints

RAC-GWVI
Research on Infectious Diseases in Gulf War Veterans

Mycoplasma infection in Gulf veterans

- Dr. Garth Nicolson first reported high infection rate by *mycoplasma fermentans* in ill Gulf veterans and family members
- Intracellular infection not detected with conventional serology, required specialized PCR methods
- He also reported these infections and multisymptom illness can be treated successfully with multiple extended courses of doxycycline, other antibiotics
- Source of mycoplasma infection? Bioweapons? Vaccine contaminant? Other?

RAC-GWVI
Research on Infectious Diseases in Gulf War Veterans

Mycoplasma infection in Gulf veterans

- Hart et al, US Army Research Institute of Infectious Diseases tested 4 lots of AVA from ODS/DS, found none contained mycoplasma DNA; mycoplasma did not survive in "spiked" samples of AVA
- Gray et al reported similar rate of symptomatic and healthy Gulf veterans test positive for mycoplasma using serologic tests, and similar rates of pre-war to post-war conversion.

RAC-GWVI
Research on Infectious Diseases in Gulf War Veterans

Mycoplasma infection in Gulf veterans

- Nicolson et al reported that 45% of symptomatic Gulf veterans test positive for mycoplasma with forensic PCR testing, compared to 9% of controls
- Vojdani et al reported that 55 % of ill Gulf vets test positive for mycoplasma species, compared to 15% of controls
  (also 49% of RA patients, 52% of CFS patients)
- Donta et al reported that 40% of ill Gulf veterans tested positive for mycoplasma when screened for recruitment into VA's antibiotic treatment trial.

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Mycoplasma infection in Gulf veterans: Major Questions

- Is mycoplasma infection associated with GWI symptoms?
  - A primary cause?
  - A cofactor?
  - An opportunistic infection, may/may not be associated with chronic symps
- Does antibiotic treatment improve GWI symptoms?
  - By eliminating mycoplasma infection?
  - By eliminating other infection(s)?
  - Through mechanisms unrelated to antimicrobial action

RAC-GWVI
Research on Infectious Diseases in Gulf War Veterans

Is Mycoplasma Associated with Gulf War illnesses?

- Several studies have found that ill Gulf veterans have a higher rate of mycoplasma infection than healthy controls
- Many questions remain
  - Problems with testing reliability and validity (sample problems, reagent differences, many years after infection, no "gold standard")
  - Prevalence of mycoplasma in healthy Gulf War veterans?
  - Is mycoplasma causally related to GWI? Is it an opportunistic infection?
  - Is there a chronic infection present? Chronic "after-effects" of acute infection?

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Research on Infectious Diseases in Gulf War Veterans

Does Antibiotic Treatment Improve GWI Symptoms?

- Summary presentation of ABT trial results generally indicated that 12 months of doxycycline was not associated with improved functional status.
- Unanswered questions remain
  - Study design:
    - Treatment protocol same as that reported to be effective by Nicolson?
    - Case definition for GWI suitable for testing hypothesis?
    - Is the primary outcome (7 pt. improvement on PCS of SF36) optimal for assessing treatment effects?
    - Confidence in laboratory identification of infected veterans?
  - Study results

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Research on Infectious Diseases in Gulf War Veterans

Infectious Disease Questions Remain

- Leishmaniasis: Detection? Prevalence of infection? Chronic sequelae of infection?
- Mycoplasma: Increased prevalence associated with illness?
- Antibiotic treatment effective in improving GWI symptoms?
- Problems due to other types of infections that have not been studied?
- Possible role for infections as a "result" of GWI, e.g., opportunistic infections, chronic immunological effects of aberrant response to infection?

Presentation 15 – Sam Donta

CSP#475 ANTIBIOTIC TREATMENT OF GULF WAR VETERANS' ILLNESSES

Background

- In 1990 and 1991, the U.S. deployed about 700,000 troops to the Persian Gulf to liberate Kuwait from Iraqi occupation.
- While there were few causalities, many individuals returned with unexplained symptoms and illnesses, which have been termed Gulf War Veterans' Illnesses (GWI).
- None of the putative etiologic agents or conditions causing GWI is supported by sufficient evidence.
- One explanation that has received widespread attention is systematic Mycoplasma fermentans infection.

Primary Study Objective

- To determine whether a 12 month course of doxycycline treatment in deployed Gulf War veterans presenting with symptoms of Gulf War Veterans' Illnesses and testing as mycoplasma positive improves patients' functional status (measured by the Physical Component Scale (PCS) of the SF-36V) compared to placebo.

Secondary Hypotheses

- To determine whether doxycycline treatment reduces symptoms of GWI including pain, fatigue and neurocognitive concerns.
- Determine whether doxycycline treatment converts mycoplasma positive patients to mycoplasma negative.
- Determine if the benefits of 12 months doxycycline treatment persist after termination of treatment.

Inclusion Criteria

- Patient deployed to Gulf (8/90 – 8/91)
- Patient has two or three of the following symptoms:
  - Fatigue
  - Musculoskeletal pain
  - Neurocognitive dysfunction
- Symptoms onset occurred during or after Gulf War
- Symptoms have occurred for at least six months and are occurring up to the present
- Patient is mycoplasma species positive (*Fermentans, Genitalium, Pneumoniae*)

Exclusion Criteria

- Medical illness capable of causing patient's symptoms
- Severe psychiatric illness
- Have received or expected to receive an organ or tissue transplant
- Requires chronic antibiotic treatment for other condition
- Life expectancy less than one year
- Known allergy to study drug

Exclusion Criteria - continued

- Patient requires phenytoin, carbamazepine, or barbiturates
- Female who refuses to use acceptable contraceptives
- Patient involved in another interventional trial
- Patient has score greater than 40 on the PCS of the SF-36V
- Patient unable to understand or give informed consent
- Patient known to have Hepatitis C

Study Overview

- This study is a 30 month, prospective, randomized, double-blind clinical trial. Patients who met all inclusion/exclusion criteria and are mycoplasma positive were randomized to one of two treatment groups:
  - Doxycycline for 12 months (200mg/day)
  - Placebo for 12 months

Primary Endpoint Measure

- Proportion of patients in each treatment group (doxycycline and placebo) with a >7 point increase in the Physical Component Scale of SF36V at 12 months, relative to baseline.

Secondary Endpoint Measures

- **Reduction in GWI Symptoms**
  - Pain: short form of McGill Pain Questionnaire
  - Fatigue: Multidimensional Fatigue Inventory
  - Neurocognitive: Cognitive Failures Questionnaire
- **Improvement in Emotional Functioning**
  - mental component scale (MCS) of SF36V
- **Mycoplasma status**
  - at 6, 12, and 18 months, relative to baseline.

Patient Intake

- **SF-36V**

Total:	Ineligible	**Eligible**
2149:	569	**1580**

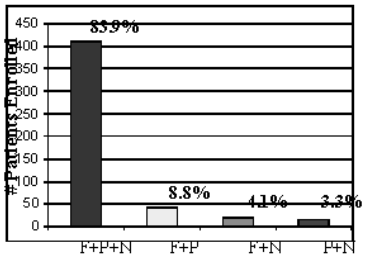
↓

Registered :**1421** → Mycoplasma PCR results: 850(-) **550(+)**

↓

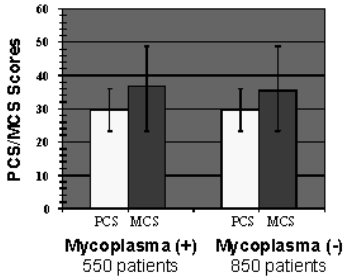
RANDOMIZED **491**

Qualifying Symptoms



F : Fatigue
P : Pain
N : Neurocognitive dysfunction

Mycoplasma Status and PCS/MCS



Compliance as Rated by Site Personnel by Treatment Group

Compliance Rating	Doxycycline		Placebo	
	#Visits	% Visits	#Visits	% Visits
Excellent	1565	57.4	1627	58.1
Good	426	15.6	458	16.4
Fair	176	6.5	174	6.2
Poor	161	5.9	197	7.0
Missing	397	14.6	342	12.2
Total	2725	100.0	2798	100.0

Number of Patients Reporting Adverse Events Considered Possibly or Definitely Related to Study Drug at Least Once During Treating Phase of Study

Costart Adverse Event	Doxycycline Group (n=245)		Placebo Group (n=246)		P-Value
	N	%	N	%	
Amnesia	21	8.6	16	6.5	0.40
Arthralgia	31	12.7	40	16.3	0.31
Asthenia	41	16.7	32	13.0	0.26
Diarrhea	40	16.3	33	13.4	0.38
Dizziness	14	5.7	9	3.7	0.30
Dyspepsia	26	10.6	20	8.1	0.36
GI Disorder	9	3.7	7	2.8	0.62
Headache	47	19.2	46	18.7	0.91
Infection	9	3.7	9	3.7	1.00
Insomnia	8	3.3	7	2.8	0.80
Myalgia	3	1.2	11	4.5	0.05
Nausea	91	37.1	25	10.2	<0.001
Pain-General	40	16.3	40	16.3	1.00
Pain-Abdomen	13	5.3	9	3.7	0.39
Pain-Back	8	3.3	13	5.3	0.37
Photosensitivity	36	14.7	15	6.1	0.002
Rash	37	15.1	27	11.0	0.18

Number of Patients Who Changed From Mycoplasma Species Positive at Baseline to Mycoplasma Species Negative at 6, 12 and 18 Months by Treatment Group

Rating Period	Doxycycline Group			Placebo Group			P
	N	# Neg	% Neg	N	# Neg	% Neg	
6 Months	206	114	55.3	213	124	56.8	0.56
12 Months	200	154	75.4	211	159	76.2	0.73
18 Months	170	154	90.6	178	154	86.5	0.25

CONCLUSIONS

- Study shows that Doxycycline is an ineffective treatment for GWVI.
- Study casts doubt on the relationship between a persistent mycoplasma infection and GWVI.
- Study documents that patients with GWVI are very ill.

Hospitalizations During Study

	Doxycycline		Placebo	
	N	%	N	%
# Patients Hospitalized At Least Once	24	9.8	33	13.4
# Patients Hospitalized For Medication Toxicity	0	0.0	0	0.0
# Patients Hospitalized For GWVI	1	0.4	5	2.0

Number of Patients Prescribed Another Antibiotic at Least once During Treatment Period

	N	%
Doxycycline	74	30.2
Placebo	101	41.1

Presentation 16 – Beatrice Golomb

Review of Recent (and recently identified) Gulf War Research

Beatrice A. Golomb, MD, PhD

R 1

Epidemiology

R 2

Australian 2003

Ss: 1456 GWV of all 1873 asked. 1588 comparison rdmly selected from Australian Defence Force that were Gulf eligible but not deployed. Queried 4-02.

Outcomes: Mental health SF12 and GHQ12. Physical health SF12. Functional impairment. # sx reported from ~61 questions.

Signif exposures: ≥10 immunizations. PB tabs. Pesticides/insecticides. Being in a CW area. AntiBW tablets. Stressful milit svc experiences.

R 3

Australian 2003

More Findings:
-↑ neuropathic sx (no dif in neuro exam): assoc with PB, solvents, pesticides, antimalarials, and immunizations.
- ↑ CFS & all fatigue-related health outcomes
- No ↑ birth defects

R 4

Australian 2003

	Dif in Phys.	Dif in Mental	OR #sx	OR Fcn†
Vaccine dose resp:	-0.5*	-0.4*	1.04*	1.1*
PB: dose resp:	-1.2*	-0.7	1.1*	1.4*
PB: any vs none	-2.5*	-2.0*	1.4*	1.8*
PB: > 250 tabs	-3.4*	-1.3	1.4*	2.5*
CW area:	-3.7*	-4.3*	1.3*	1.4*
Pesticides:	-3.4*	-3.4*	1.3*	1.5*
AntiBW tabs:	-2.3*	-2.7*	1.4*	2.1*
Repellents:	-1.1	-0.5	1.2*	1.4*
DU:	-0.2	-0.1	1.0	1.1
Deployment time‡	-0.4	-1.4*	1.1*	1.2

Phys and mental fcn from SF12. # sx from 61 sx queried.
†Functional impairment during the past 2 weeks
‡Deployment not completed before air war

R 5

Australian 2003

SF-12	Phys.	#sx	Fcn†	Mental
Vaccine dose resp:	< .001	<.001	<.001	.001
PB: dose resp:	< .001	<.001	<.001	.068
PB: any vs none	< .001	<.001	=.004	.012
CW area:	< .001	<.001	<.001	<.001
Pesticides:	< .001	<.001	=.013	<.001
AntiBW tabs:	= .001	<.001	=.01	.002
Repellents:	= .055	=.001	=.025	NS
DU:	= .718	=.939	.617	.947
Deployment time ‡	=.469	.051	=.202	.043

Phys and mental fcn from SF12. # sx from 61 sx queried.
†Functional impairment during the past 2 weeks
‡Deployment not completed before air war

R 6

Hotopf 2003: GWI better worse or same

Sample: 1245 GW: Compared to 698 Bosnia; 734 Era veterans. Stratified sampling from prior survey, based on severity of fatigue.

Outcome: self reported fatigue, Chalder fatigue scale; GHQ psych distress; SF-36 phys fcn & health perception; count of physical sx -- all are compared to response in 1997 (then N = 8196).

Finding: GWV continued to experience poorer health on all outcomes. Era vets showed lower incidence of fatigue; GWV show more persistence of fatigue than either comparator.

R 7

Hotopf 2003: GWI better worse or same

Finding:

-GWV continued to experience poorer health on all outcomes:

	GWV	Bosnia	Era
SF-36 phys	90.3->88.7	95.4->92.9	92.1->90.8
SF-36perc.	65.8->65.9	76.2->72.9	76.8->74.4
GHQ case	14.5->14.2	13.1->13.2	12.4->12.9
Fatigue	17.8->16.9	15.6->15.3	14.7->14.9
Tot #sx	11.0->10.7	6.2->7.9	5.3->6.4

R 8

Hotopf 2003: GWI better worse or same

Incidence (I) and Persistence (P). Adjusted OR

	GWV	Bosnia	Era
Fatigue>3 (I)	1.0	0.9	0.5*
Fatigue>3 (P)	1.0	0.7*	0.7*
GHQ >2 (I)	1.0	0.9	0.7
GHQ >2 (P)	1.0	1.1	0.6*
PTSD case (I)	1.0	0.8	0.9
PTSD case (P)	1.0	0.8	1.2

GHQ as index of "psychological distress"

Adjusted for:age, sex, rank, marital status

R 9

Hotopf 2003: GWI better worse or same

Incidence (I) and Persistence (P). Adjusted OR

	Era	Bosnia	GWV
Fatigue>3 (I)	1.0	1.8	2.0*
Fatigue>3 (P)	1.0	1.0	1.4*
GHQ >2 (I)	1.0	1.3	1.4
GHQ >2 (P)	1.0	1.8	1.7*
PTSD case (I)	1.0	0.9	1.1
PTSD case (P)	1.0	0.7	0.8

GHQ as index of "psychological distress"

Adjusted for: age, sex, rank, marital status

R 10

UK Gulf Mortality Data*

GW cohort: 53,409.

Era comparators: 53,143 similar age, gender, svc, rank in service Jan 1 1991 but not deployed

Outcome: deaths reported in service till Dec 31,2003

Finding:

- All deaths 0.98 (0.88-1.09)
- Disease-related death 0.82 (0.70-0.97)
- Infectious and parasitic: 1.99 (0.43-12.3)
- External injury & poisoning: 1.15 (0.99-1.35)

*www.dasa.mod.uk/natstats/natstats.html

R 11

Stability of recall of hazards over time*

Sample: 1245 GW; 698 Bosnia; 734 Era veterans stratified sampling from first survey based on severity of the fatigue and gender.

- Bosnia & GW ↑, on ave, #exposures recalled over time.
- Improved health perception was associated with ↑ "forgotten" (no longer endorsed) exposures; while worsening health perception was associated with new endorsement of exposures in Gulf but not Bosnia cohort.
- Some exposures were recalled more reliably than others, e.g. smoke from oil fire, handle prisoner of war, small arms fire, scud exploding w/in 1 mi, and seeing dismembered bodies (GW). Gulf had ↑test-retest reliability vs Bosnia.

Wessely et al Br J Psychiat 2003:183:314-22

R 12

Stability of recall of hazards over time*

- Those remaining in service were most likely to no-longer-endorse exposures, both groups.
- No-longer-endorsed is related to health perception & PTSD (not phys health or GHQ) in GWV; & to Phys health & PTSD in Bosnia.
- Newly-endorsed hazard NOT related to phys health in GWV, but was related to health perception, GHQ, PTSD (all include mental health); & related to PTSD only for Bosnia.
- Exposure vbls did not include PB; or anthrax vaccine, e.g.
- There was no assoc of exposures to health levels: phys, psychol, PTSD --but miss exposures like PB which could confound relation of other exposures

Wessely et al Br J Psychiat 2003:183:314-22

R 13

Exposures, stressors & life events

Hallman's "high symptoms" vs "low symptom" GWV were more likely to report, after adjustment (not all listed):

- BT vaccine 1.78, p = 0.02
- Anthrax vaccine 1.72, p = 0.03
- Chemical/biol warfare p < 0.01
- Days taking any PB pills, 12.0 vs 9.3%, p = 0.07
- Days taking > 3 PB pills, 3.3 vs 2.0%, p = 0.08
- Days gas mask worn ≥4hrs, p < 0.01
- Wounded , p < 0.01
- Physical deprivation , p < 0.01
- "Food/infections/equipment" p< 0.01
- Mistrust in military (.01), traumatic event (.03), "desert/exhaust (<.01)
- ...

*Adjust: age,gender, race, educ, milit branch,rank,duty,marital status, self-reported health @deployment, alcohol, smoking, illicit drug use, PTSD sx.

Boyd KC, Hallman WK et al 2003. J Occup Env Med 45:

R 14

Symptom patterns in Registry GWV

Design: mail survey completed by 1161 Registry GWV

84.5% of respondents believed they had med problems attributable to GW service;

5.3% did not answer. (~10% did not believe they did.)

Symptom list: 48 symptoms grouped by organ

* Hallman, W.K., *et al., Symptom patterns among Gulf War registry veterans.* Am J Public Health, 2003. 93(4): p. 624-30.

R 15

Symptom patterns in Registry GWV

Exploratory factor analysis: 4 symptom factors.

1. Mood/memory/fatigue
2. Musculoskeletal
3. Gastrointestinal
4. Throat/breathing

K-means cluster analysis: 2 groups

1. Healthier, 60%: ave 18 sx: 33% mod, 11% severe
2. Sicker, 40%: ave 37 sx, 40%mod, 35% severe

Cluster 2 more likely to have ≥1 of 24 medical conditions

- Includes FM, IBS, MS, CFS, depression, PTSD, bipolar, anxiety d/o, thyroid disease, DM, sterility. Hay fever, TB, eczema/psoriasis appear less frequent.

* Hallman, W.K., et al., *Symptom patterns among Gulf War registry veterans.* Am J Public Health, 2003. 93(4): p. 624-30.

R 16

Cancer in UK GWV

Sample: N=51721 GWV; N=50755 era cohort "matched" for age, sex, rank, service, level of fitness who were not deployed

Outcome: Incident Cancer

Finding: No difference in cancer.

270 GW, 269 Era cancers: Incidence RR 0.99 (.83-1.17)

Limitation: less health chosen for nondeployment & hx of illness/drugs/exposures causing or resulting from illness?

Conclusion: No evidence of excess cancer to date; merits continued follow-up due to long latency for cancers

MacfarlaneGJ 2003, BMJ 327:

R 17

Factor Analysis of Fatiguing Sx

Sample: 640 GWV with FS; 5417 GWV and 6493 nonGWV not meeting criteria & w/o exclusionary conditions. From Han Kang's 15K GWV/15K nonGWV

FS = fatiguing symptoms by 1994 mod CDC criteria x chronicity. Excess fatigue (mild/severe) first appearing in or after GW; no swelling in any joints; at least 4 of 8 sx 1st appearing in or after GW among a set; and none of a set of conditions including DM, endocrine, seizures, neuralgia, etc.

SX (4 of 8): headache, sore throat, swollen glands, muscle or joint aches/pain/cramps,fatigue lasting>24 h after exertion, awaking tired after full night sleep, difficulty concentrating/memory loss.

-- Of 11,441 GWV questionnaires, 5.6% met these criteria

Analysis: Factor analysis done separately in each group; factor correlations examined

6 subgroups/factors: named: fatigue, pain, infectious, GI, resp, & neurolog/mood/fatigue

Similar factors for each group: BUT lower interfactor correlations in GW vs control groups, lower for 13 of 15

Young HA et al 2003 J Occup Env Med 45(12)

R 18

Factor Correlations Are Lower for GW-FS

	Fatigue		
	GW.FS	GW.ctl	NonGW.ctl
	n=582	n=5076	n=6222
GI	.24	.40	.33
Resp	.13	.34	.27
Neuro	.55	.88	.81
Infectious	.14	.33	.38
Muscskel	.08	.50	.40

*Also lower for the other factors with each other

Young HA et al 2003 J Occup Env Med 45(12)

R 19

Factor Correlations Are Lower for GW-FS

Inference: More differentiated factors in GW FS group c/w distinctive set of underlying pathogenesis assoc with the factors in that group BUT subtle difs btn groups in symptom factor structures, e.g., is another possible reason. ALSO: can depend on the specific list of sx included on the questionnaire.

Importance: Different groups may have different pathogenesis and response to treatment. This approach may or may not help to differentiate such groups.

Young HA et al 2003 J Occup Env Med 45(12)

R 20

Chemical Exposures Including AChEi

R 21

Chemical Mixtures

1. PB± DEET± Permethrin causes sensorimotor deficit & change in brain AChE activity (rats).
   - Affect: AChE activity; ligand binding m2; ligand binding nicotinic rec, differ by combination and brain region. But chronic impact (after d/c exposure) not evaluated.
2. Stress +low dose chemicals damage brain areas even w/o BBB disruption (rats):
   - Some brain regions show BBB disruption (cingulate cx, dentate gyrus, thalamus, hypothalamus).
   - Regions w/o e/o BBB disruption also show effects: ↓AChE activity, ↓M2 binding midbrain/cbellum; assoc. w/ signif neuron death, ↓microtubule-assoc pr, ↑glial fibrillary acidic pr (cereb cx, HC: CA1 & CA3).

- 1.AbouDonia et al 2004. Pharmacology, Biochemistry & Behavior 77: 253-262
- 2. Abdel-Rahman A et al 2004. J Toxicol & Environ Health A 67:163-192.

R 22

AChEi: 2 sentinel patients with delayed sequela

- Case 1: 1982 IMPF & PB exposure -> myalgia & fatigue (isopropyl methylphosphonofluoridate inhalation). From 1993 developed fatigue, aches, pains esp after physical activity; also ↑CK. Nonspecific myopathy diagnoses with ragged red fibers. ↓ in concentration, memory, verbal fluency, ability to plan & initiate activities, comprehension of abstract concepts; easy distraction. Also: severe pain, digestive difficulties, weakness.
- Case 2: 1982 IMPF & PB exposure (60mg tid x 6 mo for prophylaxis) + heat stress. From early 1990s, noted myalgia, mild neuropathy, cognitive impairment, diffficulty concentrating, mood alterations and chronic fatigue. Able to do sedentary work, only 3-4h/d. CK elevation noted 1999-Jan 2003.

- Friedman L S et al 2003. CK elevations signal muscle damage following exposures to anticholinesterases: 2 sentinel patients. Arch Environ Health 58:167.

R 23

Illness Mechanisms & Markers

R 24

Sympathetic/ Parasympathetic differences

1. **Females w/ FMS or GWI show robust ↓ in HRV vs Female controls or Males (including pts) - ↓ parasymp modulation of HR. Other difs possible but small sample, n=5-19 per group.**
2. **Pts w/ CMI (chronic multisystem illness, including GWI) have ↑ catecholamine levels, Epi & esp NE, vs controls. ↓ NE response to stressor with submaximal exercise test.**

- 1.Stein PK 2003, Gender Effects on Heart Rate Variability in fibromyalgia and Gulf War Illness. 7 M GWI, 5 F GWI, 19 M control, 18 F control
- 2. Olivadoti 2003. Catecholamine responses to standardized stressors in chronic multisystem illnesses. N=53 case (5 FM, 11 CFS, 22 both, 15 GW), 36 control

R 25

Enhanced sensitivity to pain

Subjects: 12 GWV with abd pain & diarrhea s/p neg workup developed during PGW. 7 civilian & 5 veteran controls.

Exposure: a) rectal distension (35 & 55mm) & b) hot water R foot & hand (35° & 47°C x30sec)

Outcome: visual analog scale pain intensity & unpleasantness, 2 trials each

Finding: $p < 0.001$ higher rating of pain intensity and pain unpleasantness for both exposures

Conclusion: visceral hypersensitivity in PGWV with abd pain/diarrhea sim to that shown with irritable bowel. Also: cutaneous hypersensitivity "and higher levels of anxiety and somatic focus accounting for these differences in pain reporting" (no, attending them!)

Dunphy RC et al 2003, Pain 102: 79-85.

R 26

FMRI analysis of pressure pain

Subjects: 7 GWV pts, 7 FM pts, 7 healthy controls

Exposure: painful pressure to L thumb by "random staircase"; determine stimulus needed to evoke subjective mild, moderate, or intense pain. 25s blocks of painful pressure & release during 10 min FMRI sessions.

Outcome: fMRI. Pressure pain intensity.

Findings:

1. GWV&FM had ↑pressure pain sensitivity ∀ subjective levels ($p < .05$).
2. In all groups, subjective intense pain was assoc with contralateral 1° somatosensory cortex, insula, bilat. 2° somatosens cx, ipsilateral cerebellum activations. Both patient groups (only) showed activation in inf. frontal gyrus and hypothalamus not seen in controls. GW uniquely lacked activation in amygdala.

Conclusion: GWV like FM have altered pain processing: signs of pain augmentation; cerebral activations evoked by less stimulus; unique frontal and thalamic responses.

Grant M.A.B., Clauw D.J., FMRI analysis of pressure pain in Gulf War Illness, FM, and Healthy control subjects. (abstract)

R 27

Appendix B
Public Submission 1 – Dan Fahey

10 February 2004

From: Dan Fahey (duweapons@hotmail.com)
To: The Research Advisory Committee on Gulf War Veterans' Illnesses

Subj: The Need for New Research on the Health of Veterans Exposed to Depleted Uranium

The Research Advisory Committee should seek to answer the following question:

- Is the DU Program, as it is currently structured, adequately assessing the health of veterans from the 1991 war who may have internalized enough DU to cause short or long term health effects?

In my opinion, the answer to this question is "no". DoD has identified approximately 900 veterans who likely had heavy exposures to DU during friendly fire incidents, operations to recover contaminated equipment, and during and after the Doha, Kuwait munitions fire. Yet the DU Program has examined only 70 veterans in its eleven years of existence; only 58 total veterans have been examined since 1999, and in 2003 only 32 veterans were examined.

By contrast, the study of Ranch Hand veterans tracks the health of nearly 1,300 Vietnam veterans believed to have been heavily exposed to herbicides. Imagine a Ranch Hand study that included only 49 veterans – that is the same proportion as the number of DU veterans with Level I and II exposures examined bi-annually by the DU Program. The findings of the Ranch Hand study of 1,300 veterans have informed VA's decision to extend service connected benefits to veterans with diabetes, and to the children of veterans with spina bifida, but the small study size of the DU Program makes it highly unlikely that in its current form it will inform policy makers about the possible effects of DU on veterans or the need for service connected benefits for veterans and their families.

An additional problem with the DU Program is its apparent failure to fully disclose information about the health of veterans in the study. The DU Program has not publicly disclosed the development of a bone tumor in a veteran wounded by DU fragments, and the DU Program facilitated through its silence false statements made by DoD officials about the existence of cancer among veterans in the DU Program. These troubling facts call to question the integrity of the DU Program in its current form, and under its current leadership.

Recommendations:

- The Research Advisory Committee should recommend to the Secretary of Veterans Affairs that the DU Program be restructured and expanded into a cohort study that assesses the health of the approximately 900 veterans identified by the DoD as having had Level I and II exposures during the 1991 war.
- The Research Advisory Committee should recommend the expansion of research conducted by the Armed Forces Radiobiology Research Institute and other organizations to further clarify the possible health effects of DU exposure as well as the extrapolation of findings from animal studies to human populations.

Question for Albert Marshall:

- Based on your research and knowledge of DU, do you think the current size of the DU Program is adequate to make informed judgments about the health status of all veterans from the 1991 war who might have internalized enough DU to cause short or long term health effects?
    - *Follow up*: Do you think it is advisable to expand the DU Program to include health assessments of all Level I and II veterans?

Question for Terry Pellmar:

- What are the health outcomes from DU that AFRRI thinks need additional study?

Questions for Dr. Melissa McDiarmid:

- How many veterans enrolled in the DU Program have developed tumors of any kind?
    - *Follow up*: Please explain why your article in the *Journal of Occupational and Environmental Medicine* (Dec. 2001) on the findings of the 1999 round of examinations does not mention the finding of a bone tumor in a veteran who had been wounded by DU fragments.
    - *Follow up*: Given that laboratory research conducted by the Armed Forces Radiobiology Research Institute found human cells exposed to DU induced tumors when transplanted into mice, why doesn't the bone tumor in a veteran merit mention in your only official report about the health of veterans examined in 1999?
    - *Follow up*: Have you publicly reported all tumors among veterans in the DU Program?

- How many veterans enrolled in the DU Program have developed any form of cancer?
    - *Follow up*: When Dr. Michael Kilpatrick and Col. Francis O'Donnell told European press and policy makers in 2001 that no veterans in the DU Program had developed any cancers, did you make any effort to contact Kilpatrick or O'Donnell to correct the record?
    - *Follow up*: Did you make any effort to publicly correct the record by reporting the existence of lymphoma in a veteran in your study?
    - *Follow up*: Have you publicly reported all cancers among veterans in the DU Program?

- Do you believe the small size of the DU Program limits your ability and the ability of VA policy makers to draw conclusions about the health of the nearly 900 veterans identified by DoD as having had Level I and II DU exposures in 1991?
    - *Follow up*: Please explain why the DU Program examined only 32 veterans in 2003 – the fewest veterans seen since 1997.
    - *Follow up*: Do you have "customer satisfaction" surveys or other information you can provide to this committee that indicate how veterans feel about their quality of care and the value of the DU Program?
    - *Follow up*: Do you tell veterans in the study that information about their health is used to inform decisions about health care and disability benefits for hundreds of other veterans exposed to DU in past and current wars?

- Since 1993, how cooperative has the Department of Defense been in providing the DU Program with the names of friendly fire veterans and other veterans suspected of having the highest exposures to DU?
    - *Follow up*: When did you realize that there were more than 35 friendly fire veterans – the number stated by DoD until 1998?
    - *Follow up*: How many friendly fire veterans, those who rescued them, those who transported contaminated vehicles, and those at the Doha munitions fire have you attempted to enroll in the DU Program?

Public Submission 2 – Denise Nichols

Public Statement by Denise Nichols to RAC-GWVI - February 23, 2004

Deaths-Mortality - Causes--While the work of this advisory committee struggles along, another large number of Gulf War 1 veterans have died. We don't have the true death count using GWVIS data. WE need this committee to recommend that the Secretary of the VA initiate coordination with the Social Security Agency to do a match up of gulf war veterans by social security numbers and death records. The RAC should also notify congressional committees to request congressional action to get this done. Unless this is done, we will never have a true accounting of deaths that have occurred since the gulf war.

I can tell you that at least one of Dr. Haley's study subjects of the Navy Seabee group died of an apparent heart attack. Fred Willoughby was an outstanding individual known to quit a few of us here today. While we know that he is one of the Seabees with documented brain damage, we are not fully appreciating the other bodily effects that killed him and that may have impacts on the remaining gulf war veterans. Did he have a full cardiac protocol done or was that ignored?

Lab Values, Need for Research - Maybe we are being studied to death, but I believe his death must represent something to help all the rest of the ones still standing. He like the others that have died is showing other clinical indicators that are even more important! But yet no research is being done on what led to that and others deaths. Clinical - what were his lab values that were done and could we not have learned something by studying those clinical indices? What were the lab values that weren't measured, that if attention had been paid might have saved him from an earlier than expected death? Post mortem case reviews must be done and compiled into research papers. This committee needs to make that recommendation.

This past week, I helped a number of veterans. One of these has extremely high triglycerides (1,000+) and an equally elevated cholesterol level. This is not surprising to me because as the old nurse I ask the veterans about their lab work and this was not new in any way from what I had first reported to me as far back as 1993.

Need for Quality of Care Evaluation/Research - This veteran was experiencing other symptoms and went to the VA-ER. He waited 4 hours in a quiet ER before giving up and leaving. During that four hours, his complaints were minimized by the staff, his lab work was not even pulled up on the computer to be reviewed, no EKG, no follow up for holter monitoring (even through it is indicated on his record that he had a holter monitor test, in reality he has Never had one), no stress treadmill testing, no follow up cardiology appointment. I ask you is this the care you would want to receive or to know your veterans that put their life on the line for you and every other American are subjected to? Is he the next one to die for lack of appropriate standard of care? Due to the lack of the truth and critical facts that are withheld, hidden,, or ignored critical parameters are being missed.

Missing Military Records lead to Denial of help to Ill Gulf War veterans and affect the final numbers provided by the VA on ill gulf war statistics - Another veteran from this past week is one of the uncounted! He can't even get his compensation because his administrative military folder has been gutted! He is retired and getting that paycheck but it is not enough to sustain his family. He appears to have had a stroke earlier and now it seems he may have just experienced another partial stroke. He is an E7 with close to 30 years service that includes the GW. Is he one of those to come deaths that will be uncounted, unrecognized, and neglected after a life time of putting his life on the line for each American? Why is he stroking? Could it be hypercoagulation problems, Magnesium depletion, B12 depletion, unbelievable high triglycerides and cholesterol levels? Why were his military administrative records gutted, dwarfing his chances of even getting compensation much less testing and appropriate care? How many others are in this pool?

I recommend this committee immediately call for an investigation into this case and a probing to uncover the numbers of yet to be reported ill gulf war veterans in this pool of cases.

Urgent Need SPEC Scans - SPEC Scans are more commonly available that MRI-RS's, why is this test not being done on ALL Gulf War Vets? The SPEC Scans can document the neuronal cell deaths that Dr Abou Donai has researched in rats from the combination of exposures. Why hasn't the VA allowed Dr Gordon from publishing his findings in regards to all the SPEC Scans already done on GW vets? This RAC committee should recommend the research utilizing SPEC SCANS on Gulf War Veterans to be published NOW.

Need for Clinical Based Research for GW 1 Veterans - Every committee has wanted to know the effects of each potential exposure in order to prove for us what happened in reality. Will I submit that when you don't fully exam, listen for clues, see the abnormalities, review lab work and think creatively and ask for more lab work or a different workup then you are truly blind and are doing a disservice to each and every one of these veterans. While we talk of MRI-RS, and so many other sophisticated research studies, the basic clinical research parameters that are much cheaper and beneficial directly to the patient's day to day life are being hidden, ignored, or forgotten.

Communication and its effect on Care Given to GW Veterans - Area in Need of Urgent Attention: Communication to each VA hospital of the reality of the GW patients serious death prompting situation is NOT happening. The mind set of stress and psychological is killing each of us. I don't believe you die so quickly of psychological PTSD or other diagnoses that have been rendered to our GW 1 vets fighting for their lives. One of the factors that is prompting this and slowing down the true medical status of this group of veterans from being known is the absence of the true death rate and causes of death. Another factor is the withholding of information on all the known medical diagnoses of each of these gulf war veterans be it ALS, metabolic disturbances, nutritional deficiencies, MS, Cardiac, Endocrine, etc etc. All of this data needs to be put out to the public domain now. True and through investigative medical search has not occurred. Magnesium levels that have been found in at least 50% of CFS cases have not been ordered and measured in this group. The same can be said for Vit B12 and other B vitamins and so many other lab values that are not that cost prohibitive to have done. Magnesium deficiencies are also found after radiation therapies so I ask you why aren't these simple basic less costly but critical components of life sustaining treatments not being evaluated and utilized as research projects that could be done so quickly? Is it ignorance, is it purposeful intent, is it part of a systematic breakdown that started with delay, denial, and deception?

DU Testing - Today we hear more about urine testing and isotopes to find proof of DU but the simple fact is the DU excretion rate drops significantly in a time period after exposures. Backward regression type mathematical calculations would have to be performed and that has not been done. The other means would be bone biopsy where the DU has migrated. Have we learned from Gulf War 1 or are we still in denial? Why hasn't the US like the UK ordered DU urine testing immediately upon return from Operation Iraqi Freedom? The other hard proof would be chromosomal aberration testing that is within reach but not being ordered for US Gulf War Veterans! It is morally unacceptable to not utilized chromosomal aberrant testing. That testing could help in counseling veterans of the risks that they face in deciding to attempt to have children.

Other Resources - Nuclear Effects: Have we even looked at the after effects of radiation therapy to see what normal lab values become altered and applied that knowledge proactively? Have we gone back through the data gained from the radiation experiments or from the atomic veterans to gain further insight and direction for the current decade and made the announcement to clinicians of resharing of this data and direction for clinical research that would net immediate treatment modalities? Did the targeting during the gulf war not include nuclear facilities? Until ALL THE TRUTH AND DATA IS RELEASED TO THE PUBLIC, THE ATTENTION AND REDIRECTION OF DOD,VA,AND CIVILIAN MEDICAL PROVIDERS IS NOT GOING TO HAPPEN AND EACH OF US VETERANS WILL DIE EARLIER THAN WE HAVE TO!

Urgent Appeal for clinical based research or New RAC on GW Veteran Clinical Progress and Treatment - The cover of trying to find the cause or causes of the mystery undiagnosed illness and maybe a miraculous cure after a delayed time is killing us at worse or consigning us to chronic illness with lowered life fulfillment at least. The veterans are asking for day-to-day help while the search for those issues continue. I believe this committee could handle the job; after all right now you are all we have! If your charter doesn't allow these clinical based initiatives, take steps to ask for that charter to be broadened by the secretary of the VA, the President, or tell us we need to go back to the hill to have it broadened or recommend that a new Clinical Based Research and Treatment Advisory Panel for Gulf war Veterans be formed. This panel would be a panel of civilian physicians (expertise in Neurology, Immunology, Environmental medicine, and other relevant specialties), Nurses, Pharmacology Experts, and other medical health care experts, and veterans to effect day to day improvements in gulf war veterans health care now. I recommend that this be done ASAP on an urgent basis now.

Summary - In other words, simple less costly lab work and physical assessment is not being given due diligence at local VA hospitals and this could be the effects that you are searching for right before your eyes. The denials, deceptions, lack of knowledge and communication are causing misdiagnosis. These are leading to substandard medical care, medical complications, and death. While high level complicated research is going on and being funded, your sample pool (or should I say guinea pigs, or research subjects) are dying. The benefit of truly furthering comprehensive medical understanding that could benefit a civilian public facing threats of dirty bombs, biological or chemical incidents is not being gained. Remember simple correction of deficiencies shown in simple, less costly lab work can be life saving, life improving, and prove the effects that you search for in each of our bodies Now! The impact you would make by implementing clinical based research can be counted as research, just a different type, and would impact every gulf war veteran in a short time period, in a cost effective manner. WE would finally overcome to some degree the psychological over physical debate. I ask again, what abnormal basic lab work was ignored or not ordered for Fred Willoughby that could have led to simple known treatment and corrected some basic problem and prevented his early death? The time is NOW for true awareness, communication, reeducation, reemphasizing, and redirection.