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Activity of paraoxonase/arylesterase and butyrylcholinesterase in peripheral blood of Gulf War era veterans with neurologic symptom complexes or PTSD

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Abstract

Objective—Two groups of Gulf War era veterans, one exhibiting blurred vision, balance problems/dizziness, tremors/shaking, and speech difficulty and a second group with post-traumatic stress disorder (PTSD), but not the neurologic syndrome, were assessed for organophosphate-detoxifying enzyme paraoxonase/arylesterase (PON1) and its Q/R isoforms, butyrylcholinesterase (BuChE) and its U/A isoforms and cytokines.

Methods—Defibrinated peripheral blood was evaluated for enzymes and cytokines.

Results—Trends toward elevation of Th2 cytokines interleukin-4 (IL-4) and IL-13 were observed in subjects with neurologic syndrome. Neither the activities nor isoforms of the enzyme, the neurologic symptoms, nor PTSD had any relationship to wartime deployment to the theater of combat.

Conclusions—The negative outcomes described above suggest that exposure to organophosphates or other agents normally detoxified by PON1 and BuChE may not have contributed significantly to neurologic components of Gulf War Illness.

Keywords

Gulf War; interleukin-4; IL-4; interleukin-13; IL-13; Th1; Th2; neurological; post-traumatic stress disorder; PTSD; butyrylcholinesterase; paraoxonase; arylesterase; vaccines; cytokines

INTRODUCTION

Many U.S. military veterans who were activated for wartime service during 1990–1991 have developed PTSD and neuromuscular syndromes, and many also exhibit low peripheral blood

activity of enzymes, which detoxify organophosphates (compounds used as insecticides and chemical weapons). The present study evaluated two groups of Gulf War-era veterans, all of whom had completed military pre-deployment processing. One group exhibited a 4-symptom syndrome (blurred vision, balance problems/dizziness, tremors/shaking, and speech difficulty), while a second group was diagnosed with post-traumatic stress disorder (PTSD), but was not afflicted with the 4-symptom neurologic syndrome. Subjects were assessed for organophosphate-detoxifying enzyme activity and immunological function. Defibrinated peripheral blood serum contributed by each participant was evaluated for paraoxonase/arylesterase (PON1) activity and isoforms Q (active) and R (less active), plus butyrylcholinesterase (BuChE) and isoforms U (active) and A (metabolically silent). Systemic activation of pro-inflammatory immune activity was assessed by measurement of Th1-, Th2-, and inflammation-associated cytokines. Trends toward elevation of Th2-associated cytokines interleukin-4 (IL-4) and IL-13 observed in subjects with the neurologic syndrome independent of service in the Persian Gulf raise the possibility of immune contribution to neurologic symptoms resulting from pre-deployment preparation activities.

Rates of chronic illness increased dramatically among Allied military veterans of the 1990–1991 Persian Gulf War during the first decade following the conflict,¹ and continued to rise in the following years.² By mid-2008, approximately one fourth of the 697,000 U.S. personnel deployed to the Gulf were afflicted with diverse symptoms, ranging in severity from manageable to severely debilitating or fatal.^{3,4} The most frequently reported symptoms among Gulf War veterans were neurologic and neuromuscular.^{3,5} However, despite extensive studies of this phenomenon, attempts to identify objective abnormalities have produced inconsistent findings.^{5,6,7} Nevertheless, a compelling correlation between Gulf War-era military service and unambiguous anatomical changes, linked to neurologic disease, was provided by brain-scan studies of healthy Gulf War veterans, many with no combat experience or service-related injuries.

These individuals suffered a high incidence of chronic fatigue, migraines, and cognitive impairment, sufficiently severe to make normal careers and life impossible.⁸ These studies, undertaken in 2010 at Georgetown University's Center for Functional and Molecular Imaging, revealed that symptomatic veterans exhibited anomalous alterations in organization of axon bundles in both white and gray matter of regions within cortical regions of the brain known to mediate pain, cognition, and other functions corresponding to symptoms observed in these patients.⁹ By 2013, investigators conducting these and related studies reported 25% to 30% of personnel activated for U.S. military service in the 1990–1991 timeframe, and who received at least pre-combat preparation, were shown to exhibit clinically significant syndromes that include affective and cognitive dysfunction, hyperalgesia, chronic pain, and fatigue and PTSD, with definitive correlation to deterioration in axonal organization.¹⁰ Interestingly, many of these problems have been shown to result from structural alterations to the brain of affected individuals, such as increased white matter axial diffusivity.¹⁰

Large post-war increases in some autoimmune disorders were observed among Kuwaitis^{11,12} and surrounding nations.¹³ Ongoing studies of the long-term impact of exposure to military toxicants by authors of the present report on immune function have revealed significant differences in peripheral blood T cell activity in long- versus short-term residents of

Kuwait.¹⁴ The results of this initiative may converge with recent and ongoing clinical studies of U.S. and Allied military veterans¹⁵ for identification of major contributors to the pathogenesis of Gulf War-associated illness, resulting in improved strategies for prevention of and therapy of chronic illness in these populations.

Anomalous immune activity and debilitating, chronic neurologic syndromes, with anatomical correlates have persisted for decades among populations exposed to the effects of the conflict in-theater, as were residents of Kuwait as described above by Mahmoud et al in 2010 – and Rayhan et al in 2013. Moreover, the outcomes of recent studies suggest these effects will likely remain as chronic conditions for the lifetimes of many afflicted individuals, underscoring the need for continued support of efforts to analyze the phenomenon of Gulf War Illness and develop solid case definitions for the forms in which it manifests. A March 2017 report in *Journal of Medical Virology* describes common immune hyper-responsiveness and serum antibody profiles to viral proteins in Gulf War era veterans diagnosed with Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS).¹⁶ Also in March 2017, psychiatric disorders among both U.S. veterans and those serving in allied forces during the Gulf War were also reported to be elevated,^{17,15} and evidence of neurologic damage was identified in a study of U.S. Gulf War veterans conducted by investigators at Duke University and Harvard.¹⁸

Based on the outcomes of the research described above, it is possible that some disease among both veteran and indigenous populations were triggered in susceptible people by environmental cues in the wartime and postwar environment. However, the etiology of disease resulting from exposure to military toxicants during the Gulf conflict is highly speculative, and does not account for neurologic effects among U.S. veterans who served in the military during the conflict, but were not deployed to the combat theater. The experience of these individuals in the context of symptoms and laboratory correlates that they exhibit may provide enormously valuable insight into Gulf War-related illness as a result of comparison with both ill and asymptomatic persons who were exposed to the wartime environment. The clinical research on which the present report is based explores this issue.

Known high-intensity exposure sustained in-theater by veterans to chemical pollutants^{19,20} and infectious agents²¹ are likely contributors to postwar illness, including a 4-symptom neurologic factor described in the present investigation (below). These influences, along with stress of war and multiple vaccines, are known to stimulate long-lasting changes in overall patterns of immune activity that favor predominantly humoral responses to challenge by a disease-causing agent.²⁰ This mode of immune activity, which has evolved primarily as a major defense against bacteria and parasites, is characterized by high levels of antibody production and coordination by a species of T lymphocyte called a T helper-2 (or Th2) cell, along with often high expression by these cells of “signature” Th2 cytokines. A shift of immune reactivity to a predominantly Th2 pattern does not necessarily result in disease, even when induced by pollutants.²⁰ For this reason, although changes in patterns of immunological function may contribute to illness in Gulf War veterans, there is no evidence of impaired immune response in these disorders. Nevertheless, strong systemic Th2 immune bias may cause inappropriate responses to immunogenic challenge, leading to disease.²⁰ A likely case example of this phenomenon was provided by a study by Zhang *et al*, which

shows a strong correlation between cognitive impairment and Th2-related cytokine and lymphocyte profiles in Gulf War veterans with chronic fatigue syndrome (CFS), which was not observed among non-veterans with the disorder.^{22,23}

Some features of neurologic dysfunction among Gulf War era veterans appear similar to late-occurring chronic symptoms in persons exposed to organophosphates, known as organophosphate-induced delayed neurotoxicity (OPIDN).^{24–27} Gulf War-era veterans were indeed exposed to various categories and levels of occupational and environmental toxicants, principally organophosphates, in the form of pesticides and pyridostigmine bromide,^{3,4} a drug administered as prophylaxis against nerve agent, which is itself neurotoxic.²⁸ These findings notwithstanding, no clearly defined syndromes unique to veterans of the first Gulf War have yet been identified.

Management of health problems among Allied military veterans of the 1990–1991 Persian Gulf War has proven particularly frustrating since, despite claims of the existence of a “Gulf War Syndrome”, no unified case definition based on objectively characterized pathological features has emerged from studies of veterans of the conflict. Establishment of a coherent case definition is a necessary precondition for comprehensive treatment strategies in any disease. Without it, primary caregivers are forced to treat patients symptomatically and without the benefit of clear precedent.

Detailed clinical and laboratory evaluations were conducted by authors of this report, to investigate the possibility that the neurologic factor could be attributed to objectively defined pathological processes and to determine the extent to which underlying causes might resemble those for PTSD. The most intriguing result of the present study is its prominent negative outcome, specifically the apparent lack of correlation between the occurrence of the neurologic symptom cluster and organophosphate detoxification potential of the subject’s PON1 and/or BuChE isoforms.

This finding raises two interesting questions, as tens of millions of dollars have been allocated by the U.S. Veterans Administration, based substantially on a core assumption that neurologic illness in Gulf War was caused by in-theater exposures to military toxicants – and that an argument for this would be bolstered by higher rates of neurologic dysfunction among persons with reduced endogenous detoxification capacity. The present study demonstrated that, at least in the sample population evaluated here, such correlation was not present.

The major questions that emerge include, first: What aspects of pre-deployment preparations to which the subjects were subjected might have influenced development of the syndrome? The vaccination regimen given to troops during processing for combat is a possibility, particularly in the context of information given in the present report. Such a conclusion nevertheless remains speculative and will require further investigation. Secondly, the question now emerges whether these outlays represent cost-effective use of research funding? Or alternatively, was this funding allocated based on “pet” hypotheses, with only weak basis in data?

MATERIALS AND METHODS

Participants and test group designations

Participants in the present study included 53 subjects randomly selected from 30,000 respondents to a National Health Survey of Gulf War era veterans,²⁹ which was used in the original factor analysis-based determination of evidence for a deployment-related Gulf War Syndrome.³⁰ This study was approved by The George Washington Institutional Review Board (IRB#119911). Subjects participating in this study included: **i.** Persons exhibiting all 4 symptoms of the neurologic factor, and who had served in the Persian Gulf region during the timeframe of the conflict (SYM-DEP); **ii.** Persons with all 4 neurological symptoms who had served on active military duty during the conflict, but had not been deployed for service in the Gulf (SYM-NONDEP); **iii.** Persons with PTSD, but none of the 4 aforementioned neurological symptoms, who had served in the Gulf region during the conflict (PTSD-DEP); and **iv.** Randomly chosen veterans, who served in the Gulf region with no history of PTSD, or any of the four neurological symptoms (NONSYM-DEP). The subjects included 25 in the SYM-DEP group, 10 in the SYM-NONDEP group, 14 in the PTSD-DEP group, and 4 in the NONSYM-DEP group. Evaluation of all subjects for symptoms used to define each of the 4 groups was made as part of detailed medical examinations at The George Washington University Medical Center. Objective laboratory analyses for selected serum biomarkers conducted in the present study were also performed on the four aforementioned groups as described below.

Exclusion criteria

There were no exclusion criteria. The only excluded subject was a veteran with multiple sclerosis who became too ill to travel to the study site.

Experimental objectives

There were four study goals:

1. Determine the presence or absence of significant differences in serum activities of PON and BuChE between subjects with the neurological factor or PTSD and the asymptomatic controls.
2. Determine the presence or absence of significant differences in serum cytokine levels activities between subjects with the neurological factor or PTSD and the asymptomatic controls.
3. Where differences in cytokine levels exist between test and control groups, characterize the manner of change in immune function (e.g., do the results suggest a shift toward Th2 or Th2 orientation? Or is there an indication of a possibly cryptic inflammatory pathology in any of the test groups based on serum profiles of proinflammatory cytokines?
4. Determine if any findings were specific for those deployed to the theater of operations for the Persian Gulf War as compared with the smaller group of veterans with the same constellation of symptoms who did not serve in the Gulf War.

Investigative strategies and procedures

Laboratory studies—Peripheral venous blood taken from each participant was defibrinated and evaluated for two major parameters: **i.** Cholinesterases, which metabolize organophosphates and other health-impacting toxicants; and **ii.** Serum cytokines, abnormal levels of which might be indicators of incipient or active immune dysregulation and/or inflammatory processes. Since the occurrence of neurologic disorders in some veterans of the 1991 Gulf War has been reported to correlate with low activity of PON1 and BuChE,¹⁹ these enzymes were studied in the serum of participants in the present study. The samples were additionally screened for distribution of the PON1 alloenzymes: type Q, the form that most efficiently hydrolyzes organophosphates (including those used as chemical warfare agents), and type R, which is less efficient.¹⁹ Phenotypic ratios of BuChE alloenzymes, UU (the most active form), UA (a form with intermediate activity), and AA (a largely inactive form).

Serum enzyme activity—The serum activity of Paraoxonase (PON1)/Arylesterase and the distribution of the PON1 phenotypes QQ, QR and RR were measured, along with Butyrylcholinesterase (BuChE) activity and phenotypic expression of its common allele (U) and other alleles including the atypical (A)(silent) allele of this enzyme, in each of the 5 cohorts.

Paraoxonase/arylesterase assays: Serum PON activity and its phenotyping were performed using phenylacetate as the substrate. Briefly, total arylesterase activity was measured by adding 5 µl serum to 3.0 ml of solution containing 1mM phenylacetate, 9.0 mM Tris/HCl (ph 8.0) and 0.9 mM CaCl₂.^{19,31,32}

Butyrylcholinesterase (BuChE) assays: Serum BuChE activity was assayed with benzoylcholine as the substrate as described.^{33,34}

Serum cytokine levels

Cytokine measurement: Analyses were carried out at the Department of Immunology of George Washington University Medical Center, using the Luminex-100 system. In it, the concentration of serum proteins is determined by steady-state FACS analysis using capture antibody-bound, fluorophore-labeled latex microbeads. Cytokines measured include: Th1-associated IL-2, IL-12, and interferon-gamma (IFN-γ); Th2-associated IL-4, IL-5, IL-6, IL-10, IL-13, and granulocyte macrophage-colony stimulating factor (GM-CSF); and the inflammation-associated (pro-inflammatory) cytokines IL-1-β, IL-6, and IL-8.

Statistical analysis—Data analysis emphasis has been placed on descriptive statistics because of the small sample size of the asymptomatic, deployed (NONSYS-DEP) control group (n = 4). Statistically significant differences in enzyme activity between the symptomatic and PTSD groups was evaluated using the student's T test to examine group differences in serum enzyme activity levels, given as mean values ± standard deviation, with p<0.05 considered to be significant. These analyses were performed using the SPSS for Windows program version 14 (Norusis/SPSS, Inc.). As an initial conservative approach to analysis of serum cytokine levels, the results of the NONSYM-DEP group were used to

determine normal values. The highest value for each cytokine found in these serum samples was identified, with a value more than 20% higher than the highest value in the 4 asymptomatic controls as the lower limit, to be designated as “elevated” in the other three groups.

Role of the funding source—The sponsors of this investigation played no role in design of the study or in collection, analysis or interpretation of data. Nor did they participate in or writing of this report. The corresponding author had full access to all the data and had final responsibility for the decision to submit for publication.

RESULTS

Serum Paraoxonase (PON1) and Arylesterase activity

No significant differences in enzyme activity was observed between any of the subject groups (Table 1A), and distribution of PON phenotypes did not suggest correlation between any particular phenotype and occurrence of neurological symptoms (Table 1B).

Serum Butyrylcholinesterase (BuChE) activity

No significant differences in BuChE activity was observed between any of the subject groups (Table 2A), and distribution of UU and other phenotypes did not suggest correlation between any particular phenotype and occurrence of neurological symptoms (Table 2B).

Serum Cytokines

Serum concentration of representative Th1, Th2 and pro-inflammatory cytokine levels were measured in peripheral blood of participants. As described in Methods, the number and percentage of subjects in each of the 3 test groups expressing levels of each cytokine 20% or more in excess of the highest level for that cytokine in asymptomatic control group (NONSYM-DEP) was determined. This provided a qualitative picture of trends in cytokine levels that might become statistically significant in similar studies using a larger sample n for the asymptomatic control group (Table 3). As shown in figure 1, cytokine levels expressed by many subjects in all 3 test groups were observed to be at least 20% in excess of the highest level in the control group. This elevation was particularly pronounced in the case of Th2 cytokines IL-4 and IL-13 (Figure 1B).

DISCUSSION

The present study evaluated a hypothesis that neuromuscular dysfunction among Gulf War-era veterans is consistent with late-occurring chronic symptoms of OPIDN. Validation of an argument that “Gulf War Syndrome” fits an OPIDN case definition would potentially allow therapeutic strategies to be developed based on known characteristics of OP-related chronic illness. Data used as the basis for this investigation was obtained from 30,000 Gulf War era veterans, all of whom had undergone pre-deployment preparation. To characterize patterns of symptoms in these populations, the authors conducted a questionnaire-based study of 15,000 veterans of combat deployment to the Persian Gulf theater and 15,000 Gulf War-era veterans who did not serve in the Gulf.²⁹ Factor analysis of data produced by this study

identified a cluster of 4 symptoms: **i.** blurred vision, **ii.** balance problems, **iii.** dizziness, tremors/shaking, and **iv.** speech difficulty.³⁰ This cluster of four symptoms, designated a “neurologic factor”, was found in 2.4% of combat-deployed veterans and 0.45% of non-deployed Gulf War-era veterans.³⁰ Individuals with this putative neurologic syndrome also, as a group, reported elevated incidence of other symptoms, possibly indicative of underlying CNS disease, including repeated convulsions, blackouts, and seizures. These latter abnormalities were also found to be increased among veterans diagnosed with Post-Traumatic Stress Disorder (PTSD).³⁰

The results shown here failed to demonstrate a link between either PTSD or the neurologic factor and experiences of veterans during tours of duty, although the presence of some objectively defined neurological abnormalities was detected in many veterans experiencing the factor.³⁵ Significantly, the data shown here reveals that PTSD and neurological symptoms do not correlate with abnormally low peripheral blood low activity of the enzymes butyrylcholinesterase (BuChE) and paraoxonase/arylesterase 1 (PON1) (Table 1A, 1C), which detoxify organophosphates,¹⁹ or the RR PON 1 phenotype (Table 1B). These results argue against a significant contribution by toxicants, which may be efficiently hydrolyzed by PON 1 or BuChE to the etiology of the particular cluster of neurologic symptoms examined in this study. Therefore, organophosphate toxicity due to in-theater OP exposure is unlikely to be a cause of neurologic symptoms among the subjects of this investigation.

The findings presented here contrast with outcome of studies by Haley *et al*, who reported that neurologic symptoms in one population of Gulf War veterans fit an objectively measured case definition of toxicant-induced neurologic illness in one population of overseas-deployed Gulf War veterans.^{24,25} Symptoms in the veteran group studied by Dr. Haley were consistent with organophosphate exposure-associated injury to the nervous system,^{26,27} and strongly correlated with exposure to variety of influences known or suspected to cause or exacerbate disease, including organophosphate insecticides, and close proximity to possible release of chemical warfare agent in-theater.¹⁹

Further studies by Haley *et al* revealed that expression of the PON1 R allele, which hydrolyzes organophosphate (OP) compounds less efficiently than the Q form, was detected in the blood of Gulf War veterans with neurologic illness more frequently than in asymptomatic controls.¹⁹ The sample population used in these studies was very narrow, consisting of 249 subjects, comprising 41% of a single overseas-deployed naval construction battalion.^{24,36} It is thus possible that, although neurologic symptoms exhibited by these subjects were likely related to toxicant exposure and relative capacity of each individual to clear certain pollutants, their experience may not be representative of neurological disorders among Gulf War veterans as a group. One possibility is that the battalion’s in-theater mission assignment resulted in a particularly unlucky convergence of heavy toxicant exposures.

Interestingly, multiple vaccinations have emerged as a statistically valid commonality for both deployed and non-deployed veterans afflicted with the neurologic factor examined in the present report. A previous investigation reveals that although no correlation was found

between occurrence of the 4 symptom categories and health-impacting experiences while deployed in the Gulf, including exposure to OP and other toxicants, symptomatic veterans (SYM-DEP and SYM-NONDEP) had received a significantly greater number of immunizations temporally related to the Gulf War, compared with the other two groups (PTSD-DEP and NONSYM-DEP).³⁵ Although multiple vaccines are a vital component of Force Health Protection (FHP), significant adverse immune and neurological effects are associated with their use under some conditions.^{20,37–43} It is extremely important to caveat any discussion of vaccines to pathology by emphasizing their broad value in the prevention of disease.

Vaccines have a long history of safe and effective use worldwide. However, their specific effects on the immune system, in combination with war-related factors such as extreme stress and heavy exposure to environmental toxicants, have not been extensively explored. Their potential for causing systemic Th1/Th2 shifts when given at very high doses and with certain adjuvants is well documented.²⁰ Nevertheless, their high value to force health protection in environments that may include biological warfare agents makes vaccines an essential component of military medicine.

Moreover, the high potential for tactical or even strategic use of biological agents in modern warfare is a likely motivator for U.S. policy decisions to administer adjuvant-amplified multiple vaccinations as part of medical workup in preparation for deployment to combat theaters.²⁰ The present report suggests that pre-deployment vaccines shifted Th1/Th2 immune orientation in ways that contributed to the pathogenesis of neurological symptoms at some level. However, no data are presented here which demonstrate specific correlation between vaccinations and neurologic symptom complexes or PTSD.

Statements made by the authors on this topic are acknowledged to be speculative, but based on two valid rationales. First, the vaccine regimen administered as preparation for combat, included components and dosages that are well documented to have produced adverse effects in recipients, including neurological syndromes.^{20, 37, 38, 41–43} Secondly, although data presented here, clearly demonstrate correlation between occurrence of the neurological symptom cluster and having experienced pre-deployment processing, none of the pre-deployment activities with the exception of vaccination are known to impact immune or neurological status.

The potential influence of confounding factors other than vaccines was carefully considered in analysis of data shown in this report. The authors considered the possibility that lipid lowering agents, aspirins and other drugs might alter PON1 paraoxanase activities. Nevertheless, there were no exclusion criteria and data on current medication and dosage was not collected. These study design considerations were made based on the unique makeup of the subject population in 1990–91 during the time of their likely exposures. All persons completing processing for Gulf War service were of military age and were required to be in excellent health in order to qualify for deployment. Thus, the investigators considered it unlikely that a significant percentage of persons from whom data was drawn for the present study would have been receiving medical treatment sufficiently disruptive of normal homeostasis to affect outcomes shown in the report. Additionally, the exposures

reported here, were exceedingly diverse and some undoubtedly were capable of affecting PON1/paraoxanase activity to some extent. Nevertheless the fact that the participating subjects were randomly selected from the large group of 30,000 Gulf War era veterans in the national health survey^{29, 30, 35} led the authors to believe that attempting to analyze specific current medications would not be statistically justifiable.

The present study failed to demonstrate a clear correlation between the occurrence of the neurologic factor and either combat deployment or activity of enzymes capable of organophosphate detoxification. This research initiative has therefore not produced results of short-term clinical benefit to Gulf War-era veterans. These outcomes notwithstanding, this data exhibits trends in the pathophysiology of neurologic disease among Gulf War-era veterans. This may ultimately lead to sustainable strategies for long-term health management in this population. Of great interest was the observation that twice as many factor-afflicted veterans reported 5 or more immunizations temporally related to the Gulf War than did veterans in the PTSD or asymptomatic group.³⁵

Another significant finding is a possible trend towards correlation between the occurrence of neurologic symptoms and the elevation of Th2 cytokines. This is seen particularly in interleukin-4 (IL-4) and interleukin (IL-13), which are observed in the peripheral blood of subjects. This is interesting because some environmental factors contributing to neurological disease also affect Th1/Th2 immune orientation. Nevertheless, since the serum cytokine data in this study was descriptive and reliant on a very small control group, no claim is made here that the observed trend represents an incipient or active pathology. The main value of this observation is that it reinforces the need to include immunoparameters in studies of Gulf War veterans.

CONCLUSIONS

Serum and immune biomarkers observed in Gulf War era veterans with the neurologic syndrome were compared to concurrent controls and population-based data. These abnormalities were not found in PTSD. In summary, this study demonstrates that the occurrence of neurological symptoms in a representative sample of persons drawn from a large pool of Gulf War era veterans fails to correlate with the phenotype or activity of enzymes that detoxify organophosphates. The data presented here also suggest a trend toward elevated systemic expression of Th2 cytokines as a feature of the neurologic factor. These results underscore the need for a medical surveillance system in which blood products from veterans and indigenous civilian populations of combat theaters may be evaluated comparatively over time.

Future directions

Ongoing studies by the authors include a continued focus on the characterization of immunological features of chronic illness among Gulf War era veterans, with particular focus on the immunophenotype of T lymphocyte subpopulations. It is anticipated that optimization of the safety and efficacy of military vaccines will be a significant outcome of this research, along with improved case definitions of neurologic syndromes in these veterans.

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1A. Th1 Cytokines

1B. Th2 Cytokines

1C. Pro-Inflammatory Cytokines

Figure 1. Relative expression of Th1, Th2, and pro-inflammatory cytokines in peripheral blood Percentage of subjects in each cohort expressing representative Th1 (1A), Th2 (1B), and pro-inflammatory cytokines (1C) at serum levels equal to or greater than 20% higher than the highest level expressed by asymptomatic, deployed subjects (NONSYM-DEP), are designated as a negative control group. All cytokine assays were conducted using the Luminex 100 system

Table 1
Paraoxonase and Arylesterase activity and phenotypes

Activity of the paraoxonase/arylesterase 1 (PON1) gene product (1A) and distribution of the QQ, QR and RR phenotypes of the enzyme (1B) measured in defibrinated serum from subjects in each subject group.

1A. Serum Paraoxonase (PON1) and Arylesterase activity.				
	SYM-DEP Deployed, symptomatic (N = 25)	SYM-NONDEP Non-deployed, symptomatic (N = 10)	PTSD-DEP Deployed with PTSD, asymptomatic (N = 14)	NONSYM-DEP Deployed asymptomatic (N = 4)
Paraoxonase uMol/ml/min	577±81	576±124	479±107	518±248
Arylesterase uMol/ml/min	111±3	96±8	102±7	116±8

1B. PON Phenotypes (number of subjects expressing the allele in each group).			
PON Phenotype	QQ	QR	RR
SYM-DEP	10	7	8
SYM-NONDEP	2	5	3
PTSD-DEP	6	5	3
NONSYM-DEP	2	1	1

Table 2
Butyrylcholinesterase (BuChE) activity and phenotypes

Serum activity of butyrylcholinesterase (2A) and distribution of UU and other isoforms of the enzyme (UA, AA, AK, KK) (2B) were measured spectrophotometrically in a defibrinated serum from each subject group.

2A. Serum Butyrylcholinesterase activity.				
	SYM-DEP Deployed, symptomatic (N = 25)	SYM-NONDEP Non-deployed, symptomatic (N = 10)	PTSD-DEP Deployed with PTSD, asymptomatic (N = 14)	NONSYM-DEP Deployed, asymptomatic (N = 4)
BuChE activity (uM/ml/min)	0.63±0.03	0.70±0.04	0.64±0.04	0.65±0.07

2B. BuChE Phenotypes (number of subjects expressing the allele in each group).		
BuChE Phenotype	UU (most common allele)	Others (mostly A and K alleles)
SYM-DEP	19	6
SYM-NONDEP	10	0
PTSD-DEP	10	4
NON-SYM-DEP	4	0

Cytokines evaluated in peripheral blood of subject groups

Table 3

Relative expression of Th1-, Th2-, and inflammation-associated cytokines in peripheral blood of each subject group are compared with levels in the healthy control group (NON-SYM-DEP)^a. Sample n: SYM-DEP: 25; SYM-NONDEP: 10; PTSD-DEP: 14

Cytokine	Levels (pg/ml) in healthy control group ^b	SYM-DEP Number over ^c	SYM-DEP % over ^d	SYM NON-DEP Number over ^c	SYM NON-DEP % over ^d	PTSD-DEP Number over ^c	PTSD-DEP % over ^d
IL-2 (Th1)	0 – 31.2	8	42	4	40	2	20
IL-12 (Th1)	0 – 7.8	4	21	4	40	2	20
IFN- γ (Th1)	0 – 15.6	9	47	4	40	2	20
IL-4 (Th2)	0 – 31.2	10	53	6	60	2	20
IL-5 (Th2)	0 – 7.8	0	0	1	0.1	0	0
IL-7 (Th2)	0 – 9.6	4	21	1	10	2	20
IL-10 (Th2)	0 – 7.8	9	47	4	40	2	20
IL-13 (Th2)	0 – 10	10	53	5	50	2	20
GM-CSF (Th2)	0 – 16.2	7	37	4	40	2	20
IL-1 β (Inflam)	0 – 5.0	0	0	1	10	0	0
IL-6 (Inflam)	0 – 9.7	3	16	4	40	2	20
IL-8 (Inflam)	0 – 62	2	11	2	20	0	0

^aThis group of 4 subjects is made up of non-deployed veterans without neurological symptoms or PTSD.

^bThe peripheral blood serum concentration ranges for these cytokines in normal, healthy control individuals has been kindly provided by Diagnostic Products Corporation (Los Angeles, CA) and R&D System Inc. (Minneapolis, MN). Sample n for each range listed was at least 250.

^cNumber of subjects with peripheral blood levels of a particular cytokine 20% higher than highest value for that cytokine in the control group (NONSYM-DEP).

^dPercentage of subjects with peripheral blood levels of a particular cytokine 20% higher than highest value for that cytokine in the control group.