CME Article #1

Paraoxonase in Persian Gulf War Veterans

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Learning Objectives

- Summarize what previous studies have shown about possible causes of low serum paraoxonase (PON1).
- Recall what this study showed about serum PON1 activity in Gulf War veterans, in veterans deployed elsewhere, and its relation to symptoms.
- Present possible explanations of group differences in serum PON1 activity.

Abstract

Serum paraoxonase (PON1) is responsible for the metabolism of organophosphates in serum, and PON1 activity is a major determinant of their toxicity in humans. There have been reports linking lowered PON1 activity to physical symptoms after deployment to the Persian Gulf War (PGW) of 1990 to 1991. Therefore, the object of this study was to determine (1) whether PON1 activity was decreased among symptomatic PGW veterans compared with asymptomatic PGW veterans and (2) to determine whether PGW veterans as a whole had lower PON1 activity compared with other military control groups. This was a case control study nested in occupational cohort study of military personnel. Four groups of military personnel were identified from a large epidemiological study of health effects of deployment to the PGW and Bosnia: (1) symptomatic PGW veterans, n = *115; (2) healthy PGW veterans,* n = *95; (3) symptomatic Bosnia peacekeeping veterans,* n = *52; and (4) symptomatic nondeployed military controls,* n = *85. The main outcome measures were PON1 activity and genotype for PON1–55 and -192. We found significant differences in PON1 activity among these four groups, and although the two Gulf groups did not differ in PON1 activity, those deployed to the Gulf had significantly lower PON1 activity compared with the non-PGW groups (median difference = 70.9; 95% CI: 20.2, 121.5,* P = *0.012). These differences were not explained by a range of potential confounders, or differences in PON1 coding region polymorphisms. PON1 activity is reduced in PGW veterans compared with military control groups. The effect is independent of ill health in PGW veterans.* (J Occup Environ Med. *2003;45:668–675*)

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Matthew Hotopf has no commercial interest related to this article.

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DOI: 10.1097/01.jom.0000071506.96740.39

A wide range of candidate exposures have been suggested as possible causes of ill health in Gulf War veterans (GWVs). Most studies have found nonspecific effects with many diverse exposures being associated with ill health.[1] However, there have been some reports that exposure to chemical agents, such as organophosphate (OP) pesticides, *N*,*N*,-diethyl-m-toluamide (DEET) insect repellent, and pyridostigmine bromide, might be more specifically associated with neurological syndromes.[2,3]

The hydrolysis of OPs by serum paraoxonase (PON1) is a major determinant of their toxicity to vertebrates, including humans.[4–9] In PON1 knockout mice, the absence of the PON1 gene results in rapid death on exposure to OPs in doses sublethal to wild-type mice.[9] In the humans, there is considerable individual variation in the serum activity of PON1, and this is partly genetically determined.[4,7,8] Two genetic polymorphisms in the PON1 coding region caused by amino-acid substitutions at position 55(L→M) and 192(Q→R) result in PON1 isoenzymes, which differ greatly in their activity toward various substrates[10–12] The specific activity of the alloenzymes created by the 192 polymorphism depends upon the nature of the OP substrate.[13] Diazoxon, the metabolite of diazinon, is metabolizesd more slowly by the R alloenzyme, whereas other OPs, such as paraoxon, are metabolizesd more slowly by the Q alloenzyme.[11] Such differences in OP hydrolysis, which might lead to differential susceptibility to OP toxicity, have been found to be associated with ill health in those who have been chronically exposed

to OPs, for example, sheep farmers.[14]

Haley and coworkers reported a case control study of 25 US GWVs with neurological symptoms compared with 10 healthy GWV controls and 10 military controls not deployed to the Gulf.[15] The participants were selected from a cross-sectional study with a participation rate of 41%. The study found an association between the PON1-Q allele and neurological symptoms, suggesting that those who were ill had a genotype that led to lower PON1 activity and thus greater vulnerability to OP toxicity. A subsequent British case control study[16] selected 152 ill GWVs from the database of a veterans' association and 152 healthy civilian controls attending health screening who were matched for gender and age. Serum paraoxon hydrolysis and PON1 concentration were significantly lower in the veterans compared with controls. However, the distribution of the PON1–55 and 192 polymorphisms were not different between the groups.

Low PON1 activity is associated with several diseases that have an inflammatory component, such as coronary heart disease, diabetes mellitus, and renal disease (reviewed in the references[12]). One explanation might be, therefore, that GWVs have chronic inflammation possibly caused by the immunization program or some other factor unique to the Gulf. Both acute and chronic inflammation reduces the hepatic synthesis and secretion of PON1,[17] which is mediated by proinflammatory cytokines, such as tumor necrosis factor-α and interleukin (IL)-6.[18] We therefore aimed to determine whether any lowering of PON1 activity in PGW veterans could be explained by proinflammatory cytokines.

The present study aimed to replicate the findings of previous studies using an epidemiologically robust study design. We tested the hypothesis that compared with healthy GWVs, ill GWVs would have higher rates of the PON1-Q allele or decreased serum activity of PON1. Furthermore, we tested the hypothesis that this effect would be specific to ill GWVs, and two control groups of military personnel who were symptomatic but had different deployment histories would not show such effects. Finally, we determined whether service in the Gulf, irrespective of health status, had an impact on PON1 activity.

Methods

Study Design

Approval was gained from our local research ethics committee. A two-phase study design was used. Phase 1 was a population-based postal health questionnaire survey comparing self-reported health indices in three randomly selected cohorts of UK Armed Forces. The cohort of interest was those deployed to the Gulf conflict between 1st September 1990 and 30th June 1991. We chose two control cohorts; those who served in Bosnia as part of the United Nations peacekeeping forces between 1st April 1992 and 6th February 1997 ($n = 3450$) to match for the experience of being deployed and a nondeployed group defined as those who were in active service in the UK Armed Forces during the Gulf conflict but not deployed to the Gulf (Era; $n = 4248$) to match for military experience. The methodology, response rates, and main findings of phase 1 have been published elsewhere.[1] In phase 2 (reported here), Gulf veterans who in the phase 1 screened positive for physical disability were compared first with Gulf veterans who screened negative and then with Bosnia and Era veterans who screened positive for physical disability.

Definition of Participants

In the absence of a clear definition of Gulf-related ill health, we used a generic measure of physical disability, the Short-Form 36 Physical Functioning subscale (SF-36 PF),[19] which was measured at phase 1 as our proxy measure for ill health. The value of the first decile of the distribution of the SF-36 PF in the Era cohort (score = 72.2) was used as the cut-off below which we defined disability in all three cohorts. The Era cohort was considered the most representative of the military as it represented 80% of the UK Armed Forces. The rationale for using a generic measure was to allow a comparison between Gulf and non-Gulf (Bosnia and Era) veterans that would be unbiased by different distributions of symptoms or assumptions about the nature of ill health in Gulf and non-Gulf veterans. This definition of ill health was designed to identify participants whose disability was most likely to be clinically significant. Gulf veterans who reported impaired physical functioning below the cut-off were defined as disabled Gulf with those above the cut-off defined as nondisabled Gulf. Bosnia and Era veterans who reported impaired physical functioning below the cut-off were defined as disabled non-Gulf. Bosnia and Era veterans were sampled separately from their respective cohorts but were grouped together in some of the statistical analysis to form one group that would be more similar to the Gulf cohort. The numbers of disabled and nondisabled Gulf and disabled Bosnia and Era veterans that were eligible to participate and from which randomly selected samples were drawn was 406, 3047, 138, and 278 veterans, respectively. We used computer-generated random numbers in batches of 100 for each group. If, after random selection, the subject was deceased or reported a currently diagnosed serious physical illness, he/she was excluded and replaced by another randomly selected individual from the remaining eligible samples. Participants were invited to attend the Gulf War Illnesses Research Unit at King's College Hospital, London for a 1-day medical assessment between January

1999 and September 2000. Local ethics committee approval for the study was given and informed consent was obtained.

Biochemical Analysis

The biochemical analyses were performed masked to the participants' illness status and deployment. Serum hydrolytic activity toward paraoxon, was determined as described in a previous study.[20] After DNA extraction, PON1–55 and -192 genotype were determined by polymerase chain reaction and restriction enzyme digestion using standard published protocols.[16,20] Plasma apolipoprotein AI (apoAI) and high-density lipoprotein (HDL) were measured using a Cobas Mira S autoanalyser with reagents and standards supplied by the manufacturer (ABX Diagnostics, Shefford, Bedfordshire, UK). These assays were performed because PON1 is transported on HDL.

Immune Assays

Flow cytometry was used to measure intracellular cytokine production by CD4 T lymphocytes. Heparinized venous whole blood taken from all subjects at approximately the same time of day to avoid the effect of diurnal variation and was mixed with tissue culture medium (TCM; RPMI 1640, 10% fetal calf serum, 2 mM L-Glutamine, 100IU/mL penicillin, 100 μg/mL streptomycin, Life Technologies, Paisley, Scotland) in the proportion 1:3.5 TCM. One-milliliter aliquots of cell suspension were then either supplemented with polyclonal activators to 5 ng/mL final concentration of M phorbol 12-myristate 13-acetate and 745 ng/mL of ionomycin (Sigma Chemical Co, Poole, UK; stimulated cells), or incubated without polyclonal activators (nonstimulated). Protein secretion inhibitors brefeldin A (5 μg/mL) and monensin (2.08 mg/mL; both Sigma) were then added to all cultures, which were incubated at 37°C for 16 hours in 5% CO_2.

Cells were harvested, washed, and stained with PerCP-conjugated anti-human CD4 monoclonal antibodies (mAb) (Becton Dickinson, San Jose, CA), then washed twice with phosphate-buffered saline containing 5% fetal calf serum and 0.01% sodium azide and intracellular cytokine staining performed as suggested by the manufacturer (Fix&Perm Permeablilization Kit, Caltag Lab, Burlingame, CA). In brief, the cells were fixed with Reagent A and incubated for 15 minutes at room temperature in the dark. After washing twice, permeabilizing Reagent B was added along with appropriate PE-conjugated anti-cytokine (IL-2, interferon [IFN]-γ, IL-4, IL-10) mAbs or isotype-matched control mAbs (all Becton Dickinson) and incubated in the dark at 4°C for 30 minutes. Finally, the stained cells were washed and suspended in 200 μL of phosphate-buffered saline containing 0.01% sodium azide for flow cytometry analysis. Additional studies were conducted to examine coexpression of IL-4 and IFN-γ using CD3-FITC, CD4-PerCP, IFN-γ-PE, and IL-4–APC mAbs.

Lymphocytes were gated on the basis of forward and side scatter properties and fluorescent channel dot plot quadrant statistics set on the basis of corresponding isotype-matched control mAbs to determine the frequencies of CD4 T cells producing IL-2, IFN-γ, IL-4, and IL-10. Contamination by CD14+ monocytes in the gated CD4 population was routinely assessed as <1%. Stained cells were analyzed on a FACSCalibur cytometer using CELLQuest software (Becton Dickinson, San Jose, CA) and cytokine-positive cells expressed as percent positive of total lymphocytes.

Statistical Analysis

The initial statistical analyses were performed masked to the participants' illness status, and performed independently by VN and MH, using STATA. These initial analyses sought to replicate the analyses in Mackness et al.,[16] except four groups were compared instead of two. Analysis of variance was used to compare mean values of normally distributed variables. For skewed data, we report median values and performed a log transformation before performing analysis of variance. Chi-square tests were used for categorical data. Subsequent statistical analyses were unmasked because we wanted to make specific comparisons between those who were asymptomatic and those who were well, and those who served in the Gulf versus those who were deployed elsewhere. Multiple quantile regression analysis was carried out to assess differences in median PON activity between the four groups along with their 95% confidence intervals.

Results

Seven-hundred forty cohort members were eligible for the study. Contact was made with 607 cohort members. Nonparticipants were defined as those who refused to participate ($n = 176$) and those who initially agreed to participate but did not attend ($n = 89$). The participant rate from phase 1 to phase 2 was 66.9% for disabled Gulf, 62.4% for nondisabled Gulf, 55.1% for disabled Bosnia, and 42.5% for disabled Era groups. The characteristics of participants and nonparticipants have been described elsewhere.[21] Apart from a statistically significant ($P = 0.005$) difference in participation according to group status, participants had lower levels of psychological distress measured on the general health questionnaire (GHQ-12)[22] at phase 1 ($P = 0.03$), and were more likely to have left the armed forces ($P = 0.06$).

Table 1 describes the demographic characteristics of the four groups of participants. There were significant differences in the distribution of age, service status and rank among the groups. Those in the Era group are more likely to be older, have left the forces and belong to a higher rank. Also, those in the Gulf group who

TABLE 1
Demographic Characteristics of the Four Groups

	Gulf Well (n = 95)	Gulf III (n = 115)	Era III (n = 85)	Bosnia III (n = 52)	Statistic
Age in years					F = 15.5 df = 3
Mean (SD)	34.3 (5.4)	36.9 (7.3)	39.8 (8.5)	31.7 (6.9)	$P < 0.001$
Gender					χ^2 = 0.68 df = 3
Males (%)	85 (94.44)	100 (94.34)	70 (92.11)	46 (92.0)	P = 0.87
Females (%)	5 (5.56)	6 (5.66)	6 (7.89)	4 (8.0)	
Service status					χ^2 = 23.8 df = 3
Active (%)	50 (55.56)	32 (30.19)	23 (30.26)	30 (60.0)	$P < 0.001$
Discharged (%)	40 (44.44)	74 (69.81)	53 (69.74)	20 (40.0)	
Rank					χ^2 = 15.35 df = 3
Officer (%)	13 (14.44)	4 (3.81)	10 (13.33)	3 (6.0)	P = 0.02
NCO (%)	63 (70.0)	75 (71.43)	57 (76.0)	33 (66.0)	
Private (%)	14 (15.56)	26 (24.76)	8 (10.67)	14 (28.0)	
GHQ-12* at stage 1					F = 12.52 df = 3
Mean (SD)	3 (3.59)	6.24 (4.02)	4.09 (3.79)	3.68 (3.67)	$P < 0.001$
Total symptoms at stage 1					F = 40.6 df = 3
					$P < 0.001$
Mean (SD)	10.05 (7.8)	22.02 (10.6)	10.5 (8.1)	8.5 (8.5)	

* General Health Questionnaire.

TABLE 2
Median PON1 Activity, Mean Apo-A1, HDL, and Genotype Distribution in Each Cohort

	Gulf Well (n = 95)	Gulf III (n = 115)	Era III (n = 85)	Bosnia III (n = 52)	Statistic
PON1 activity					F=2.81,3df,P=0.03
median	168.92	145.85	222.98	216.08	
(Range)	(59.3–521)	(48.3–423)	(54.6–539.6)	(54.4–425.7)	
192 genotype					
QQ	49.4	56.7	48.5	46.8	χ^2=4.7, 6df,P=0.6
QR	43.4	35.1	48.5	46.8	
RR	7.2	8.3	3.0	6.4	
55 genotype					
LL	33.3	48.5	39.4	36.2	χ^2=11.2,6df,P=0.08
LM	61.9	41.2	45.5	53.2	
MM	4.8	10.3	15.2	10.6	
HDL	1.45	1.31	1.31	1.34	F=2.68,3df,P=0.05
Apo-A1	179.7	174.0	176.2	170.8	F=0.65,3df,P=0.6

Apo-A1, apolipoprotein A1; HDL, high-density lipoprotein; PON, paraoxonase.

are disabled have had higher levels of psychological distress on the GHQ-12 and more symptoms at stage 1. If we compare those who deployed in the Gulf with those deployed elsewhere, the only difference we found is that the Gulf group was more symptomatic (mean difference = 6.62, 95% CI = 4.27–8.97). There was no association between PON1 activity and gender or age (F(1283) = 0.01, P = 0.92; F(35,283) = 0.72, P = 0.88, respectively).

Differences Among Groups

Table 2 shows the descriptive statistics for PON activity, apoAI, HDL, and the two 192- and 55-genotypes by the four groups. There was a statistically significant difference in PON1 activity and HDL between the four groups. There were no differences in the distribution of genotypes between the four groups.

Differences Between Gulf-III and Gulf-Well Groups

Comparing the two groups of Gulf veterans, the Gulf ill group had slightly lower PON1 activity, but this did not reach statistical significance (median difference 23.1; 95% CI = −27.7, 73.9). The Gulf well group had somewhat higher HDL (mean difference 0.14, 0.04, 0.25). There were no statistically significant differences between the two Gulf groups for the PON1–192 genotype (chi-square = 1.30, 2df, P = 0.52); however, there was a statistically significant difference in the distribution of the 55 genotype (chi-square = 8.06, 2df, P = 0.02), with a higher proportion of the Gulf well group having the LM genotype.

Differences Between Those Deployed to the Gulf and Bosnia/Era Group

There was a significant difference between those who were deployed to the Gulf and those deployed elsewhere for PON1 activity (median difference = 70.9; 95% CI = 20.2, 121.5, $P = 0.012$) with the Gulf group having lower values. There were no significant differences in HDL or PON1–192- or 55-genotype between these two groups.

Genotype Interactions

To determine whether the difference in PON1 activity between groups could be caused by differences in the distribution of genotypes, Table 3 shows the distribution of PON1 activity, apo-A1, and HDL across the four groups by the three polymorphisms of 192-genotype. The table demonstrates the expected general pattern of variability of PON1 activity according to 192 genotype as would be predicted by previous work,[23] although in Bosnia Ill group there was no such pattern, possibly because of small numbers. After adjusting for genotype at position 192, there was still significantly lower PON1 activity for those in the Gulf groups compared with the Bosnia/Era groups (median difference 39.1; 95% CI = 3.09, 75.2 $P = 0.033$). Table 4 shows the distribution of PON1 activity, apoA1, and HDL according to polymorphisms of the 55-genotype. There was no consistent variation of PON1 activity according to 55-genotype polymorphism, which was not unexpected given that this polymorphism only explains 6% of the variation of PON1 activity.[20] After adjusting for genotype at position 55, there was still significantly lower PON1 activity for those in the Gulf groups compared with Bosnia/Era (median difference 60.2; 95% CI: 13.8, 106.6 $P = 0.013$). There were no significant genotype by group interactions (F(2286) = 0.10, $P = 0.91$ at position 192; F(2287) = 0.70, $P = 0.49$

TABLE 3
Mean PON Activity, Apo-A1, and HDL by 192-Genotype and Cohort

	Gulf War			Gulf III			Era III			Bosnia III		
	QQ (n = 41)	QR (n = 36)	RR (n = 6)	QQ (n = 55)	QR (n = 34)	RR (n = 8)	QQ (n = 32)	QR (n = 32)	RR (n = 2)	QQ (n = 22)	QR (n = 22)	RR (n = 3)
PON activity												
Median	145.8	173.04	293.8	125.05	178.8	254.09	138.9	270.6	364.3	240.6	216.1	199.4
(range)	(64.4–465.7)	(59.3–468.2)	(119.2–427.3)	(48.3–423)	(77.6–393.4)	(85.12–418)	(54.6–401.7)	(66.9–428.4)	(300.8–427.9)	(54.5–425.7)	(88.1–405.4)	(147.2–280.7)
Apo-A1												
Mean ± SD	174 ± 28.7	181.3 ± 30.68	171.8 ± 23.4	169.7 ± 40.5	177.3 ± 38.28	189.7 ± 30.4	182.9 ± 64.9	160.2 ± 21.47	250.0 ± 41.01	175.27 ± 36.06	166.2 ± 19.24	172.0 ± 41.9
HDL												
Mean ± SD	1.38 ± 0.32	1.48 ± 0.38	1.40 ± 0.31	1.26 ± 0.35	1.36 ± 0.48	1.46 ± 0.36	1.39 ± 0.64	1.15 ± 0.25	2.05 ± 0.14	1.36 ± 0.26	1.34 ± 0.22	1.16 ± 0.32

Apo-A1, apolipoprotein A1; HDL, high-density lipoprotein; PON, paraoxonase.

TABLE 4
Summary Statistics for PON Activity, Apo-A1, and HDL by Group and 55-Genotype

	Gulf War			Gulf III			Era III			Bosnia III		
	LL (n = 28)	LM (n = 52)	MM (n = 4)	LL (n = 47)	LM (n = 40)	MM (n = 10)	LL (n = 26)	LM (n = 30)	MM (n = 10)	LL (n = 17)	LM (n = 25)	MM (n = 5)
PON activity												
Median	249.5	137.0	242.5	161.9	151.6	115.3	273.1	145.8	147.4	227.8	204.3	252.5
(range)	(68.5–427.3)	(59.3–468.2)	(64.4–258.5)	(82.8–418)	(48.3–423)	(71.8–301.3)	(54.6–427.9)	(56.5–428.4)	(94.7–379.6)	(54.4–425.7)	(88.1–405.4)	(171.2–346.6)
Apo-A1												
Mean ± SD	175.1 ± 22.7	178.9 ± 34.2	182.5 ± 11.7	176.6 ± 36.06	172.7 ± 46.3	174 ± 26.9	172.2 ± 41.1	168.2 ± 27.3	196.6 ± 103.7	176.1 ± 33.3	167.4 ± 26.4	170 ± 32.3
HDL												
Mean ± SD	1.43 ± 0.28	1.44 ± 0.39	1.34 ± 0.20	1.35 ± 0.44	1.29 ± 0.41	1.3 ± 0.24	1.28 ± 0.42	1.21 ± 0.35	1.59 ± 0.93	1.32 ± 0.27	1.35 ± 0.23	1.34 ± 0.24

Apo-A1, apolipoprotein A1; HDL, high-density lipoprotein; PON, paraoxonase.

at position 55). This means that group differences in PON1 activity and ill health were not due to genotype. All the populations were in Hardy-Weinberg equilibrium for both the PON1 55 and 192 genotypes, with the exception of the Gulf Well group, where PON1–55 polymorphism was in disequilibrium (chi-square = 10.2, 1df, $P < 0.01$). The reason for this is unclear but could be the result of random error.

Further Confounders

Finally, we sought to determine whether the difference between the Gulf group and Bosnia/Era groups was caused by the possibility that the Gulf groups as a whole were more severely symptomatic than Bosnia/Era groups. After controlling for total physical symptoms and total general health questionnaire score at stage one there was still a significant difference between the groups (median difference 73.6; 95% CI = 33.8, 114.5).

Relationship Between PON1 Levels and Vaccine Records and Intracellular Cytokines

We hypothesized that PON1 activity might have been related to inflammatory processes in those deployed to the Gulf. We first attempted to determine among the Gulf groups whether there was any relationship between PON1 activity and total vaccines received as reported in stage 1 of the survey. We were unable to find any such association (results not shown). We also attempted to determine whether PON1 activity was associated with any of the intracellular cytokines measured, but again found no association (results not shown). Specifically, the adjusted difference in median PON1 activity between those deployed to the Gulf and those deployed elsewhere was 78.3 (95% CI = −28.8, 185.5 $P = 0.15$). Although the result was no longer statistically significant, the effect size was similar to those reported above. The loss of statistical significance is due to the fact that cytokine values were not available for the total sample, and statistical power therefore fell. Thus, intracellular concentrations of these cytokines did not explain the observed differences in PON1 activity between groups.

Discussion

The two main findings of this article are: (1) that there are no differences in the genotype or PON1 activity between healthy and ill GWVs; and (2) the participants who were deployed to the Gulf had lower median PON1 values than the other two groups, and these differences were not explained by differences in the genotypes between groups.

Our study has major advantages over the two previous studies on PON1 and deployment to the Gulf. We reduced potential selection biases by nesting the study within a cross sectional survey, and randomly selecting participants from stage 1 to stage 2. Sample sizes were large, and we used military control groups. All three armed services were represented, and sample selection included veterans irrespective of whether they were still serving. Further, the biochemical analysis and much of the statistical analyses were performed masked to group status.

Nonetheless, there were methodological shortcomings. As in most surveys of young and predominantly male populations, our participation rates were less than optimal. Because the second stage of the survey was designed to determine patterns of disease between similarly disabled veterans from the Gulf, era, and Bosnia we did not include healthy veterans from the latter two groups.

We were unable to find any statistically significant difference in PON1 activity between the two Gulf groups, but we did find that there was a slight difference in the distribution of the 55-genotype with more heterozygotes in the Gulf-ill than the Gulf-well groups. However, our main finding was of a significant difference in PON1 activity between those deployed to the Gulf and the other two cohorts. This was not explained by differences in the genotypes between these groups, nor was it caused by the two Gulf groups being more symptomatic. We therefore interpret this difference as being the result of deployment rather than illness status. We suspect that those who served in the Gulf were exposed to a specific hazard that led to a long-term decrease in PON1 activity. Possible candidates are the insect repellent (DEET); pyridostigmine bromide used as an antidote to threatened chemical weapon attack; and organophosphate pesticides. Because most of the Gulf-related hazards are specific to those who served in the Gulf, it is not possible to determine whether exposure to these would explain intergroup differences.

Studies conducted by some of the present authors have indicated that sheep farmers with long-term exposure to organophosphate-based sheep dip are more likely to have symptoms of ill health, which the farmers believed was caused by OPs, if they had the genetic isoform of PON1 least able to metabolizes diazoxon, the active metabolite of the sheep dip.[14] Furthermore, we have shown that acute exposure to OPs, taken in suicide attempts, leads to an initial loss of PON1 activity. However, activity levels recover within 6 months of the acute event.[24] Furthermore, the doses of OPs involved in these acute exposures are likely to be far higher than those experienced during the Gulf war. There are no available data on the effect of chronic exposure to OPs on PON1 activity. Nor is there any available evidence on the effect of DEET or pyridostigmine on PON1 activity. Further studies in these areas are warranted.

Our present results did not support the hypothesis that multiple vaccines accounted for the lowered PON1 activity. Nor did we find any differences between the groups in intra cellular cytokines. Although previ-

ous research indicates that increased levels of circulating proinflammatory cytokines appear to depress PON1 activity.[17,18,25] In contrast, our study measured intracellular cytokine activity in T cells. It is probable that circulating levels of pro-inflammatory cytokines are not strictly comparable to T cell secretion, since other immune cells, such as macrophages, contribute significantly to the secreted levels.

Low PON1 activity in veterans of the Persian Gulf War could also have arisen because of an overrepresentation of polymorphisms in the promoter region of the PON1 gene coding for low levels of this enzyme[26] rather than the exonic polymorphisms investigated in the present study.[27] This, in turn, could lead to a pro-inflammatory state as described above. This possibility is currently under investigation in our laboratory.

To conclude, our findings suggest that military personnel deployed to the Persian Gulf War have lower than expected PON1 activity. This effect appears to relate to the deployment per se rather than illness. The illness could therefore be the result of unidentified exposures in a group rendered susceptible by their diminished PON1 activity. PON1 has an enormous range of potential substrates in addition to organophosphates.[8] Our findings give some credence to the hypothesis that susceptibility to such symptoms may have arisen because of some exposure specifically related to service in the Persian Gulf or to the preparation of military personnel for service in the Persian Gulf. Future studies might investigate this further by studying military personnel from countries other than the United Kingdom; by comparing personnel prepared for active service but not actually deployed with those who were deployed; and by investigating the relationship between low PON1 activity and the promoter polymorphism of the PON1 gene, which might be the site of the interaction with chemical or immunological exposures.

Acknowledgments

We thank the Gulf War Illness Unit at the U.K. Ministry of Defense for their assistance in identifying the cohorts and tracing them and the veterans who participated in this study. This study was funded by the US Department of Defense.

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