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TITLE: The Role of Th1/Th2 Cytokine Balance in Gulf War-Related Illness

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13. ABSTRACT ***(Maximum 200 Words)***

The aetiology of Gulf-War (GW) related illnesses remains unclear. The proposal has been made that multiple vaccines, given under the stress of deployment with additional effects of the T helper 2 (Th2) adjuvant pertussis could skew the immune response towards a Th2 profile. The Th2 profile, characterised by the cytokines interleukin-4 (IL-4) and IL-10, has been associated with syndromes such as allergic disease, hypersensitivity and depression, symptoms characteristic of which are often reported by sick GW veterans (GWVs). We are studying whether self-reported illness in GWVs is associated with a Th2 shift by examining Th2 (IL-4, IL-10) and Th1 (IL-2, interferon-γ) intracellular cytokine staining in CD4 and CD8 T lymphocytes by flow cytometry after mitogenic stimulation. To date we have recruited GWVs with and without illness, as well as a group of control servicemen with similar symptoms who were either not deployed (era controls) or deployed to the Bosnia conflict and healthy laboratory control subjects (LCs). We find changes in cytokine profiles in each of the 3 service groups compared with the LCs. Our data suggest that deployment to the Gulf and multi-symptom illness are associated with immune activation, with different patterns seen in sick and well veterans of the Gulf conflict. We await the full acquisition of clinical and vaccine record data, to determine the clinical correlates of our immunological findings.

14. SUBJECT TERMS
Persian Gulf War; immunology; T helper subsets; cytokines; vaccines

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Introduction

In this study, we set out to examine the relationship between immunological markers of Th1/Th2 cytokine balance, biological warfare vaccines and Gulf War related illness.

Soon after their return from deployment, it became apparent that members of the armed forces who served in the Persian Gulf War in 1990 were reporting a number of unexplained illnesses, including symptoms typical of the chronic fatigue syndrome (CFS). In order to clarify whether such an increase in morbidity was real, and the precise nature of the symptoms involved, we undertook an epidemiological study of the prevalence of unexplained illness in the population at risk using a two stage design. This work, which was also US AMRMC funded, reported its first stage findings in 1999. These findings were the result of a questionnaire based study in which we compared 2961 United Kingdom service personnel who served in the Gulf conflict with 2620 who served in Bosnia, and 2614 who were in the military during the time of the Gulf War but served in neither theatre ("Era"). All were chosen randomly. The results (Unwin et al, 1999) showed that service in the Gulf was associated with a range of adverse, self reported outcomes. Symptoms such as fatigue, irritability, headache and difficulties with sleep were about three times commoner in the Gulf group. Although we were unable to confirm that these results indicated a unique "Gulf War syndrome" (Ismail et al, 1999), we concluded that this was unequivocal evidence that Gulf service had led to a subjective decline in well being and symptomatic ill health amongst those who served in that theatre.

The next question we addressed was possible aetiology. This was examined using the same cohorts, and accessing their self-reported exposure to potential aetiological agents, but especially vaccines. We found (Hotopf et al, 2000) that receipt of multiple vaccines during deployment was associated with 5 out of 6 disease outcomes (multi-symptom illness (CDC defined) fatigue, psychological distress, health perception and physical functioning but not post-traumatic stress reaction), with the strongest association for multi-symptom illness (odds ratio 5.0). Since administration of multiple vaccines *before* deployment was only associated with one outcome (post-traumatic stress reaction), our results supported the concept that multiple vaccinations combined with the stress of deployment may be associated with adverse health outcomes.

These findings are consistent with the so-called "Rook and Zumla hypothesis", namely that multi-symptom illness in war veterans is the result of a shift in cytokine balance from T helper 1 (Th1) to Th2 (Rook & Zumla, 1997). The hypothesis is based on a number of observations. First, GWVs received multiple vaccinations administered within a short space of time. It is well documented that antigen load can deviate the immune response towards Th2 (Bretscher et al, 1992; Hernandez-Pando et al, 1994). Second, vaccines were administered in preparation for war, and included those against biological warfare agents such as *Bacillus anthracis* (anthrax) and *Yersinia pestis* (plague), which were very real threats during this conflict. It is propososed that the resultant high levels of stress in vaccine recipients leads to elevated levels of circulating levels of the hormone cortisol, which has been shown under many experimental conditions to

deviate the immune response towards Th2 (Wu et al, 1991; Brinkman et al, 1995; Padgett et al, 1995; Ramirez et al, 1996). Finally, although natural infection with *Bordetella pertussis* and its whole cell-derived vaccine promote a strong Th1 response, paradoxically the acellular vaccine component pertussis toxin used as adjuvant in GW vaccinations causes Th2 deviation (Munoz et al, 1990; Mu et al, 1993). These proposals take on a greater significance when considering the symptoms and immunopathological findings in chronic fatigue syndrome, which bear many striking similarities with Gulf War associated illness and could be interpreted as representing a switch to a Th2 cytokine profile as has been suggested (Rook & Zumla, 1997).

The Th1/Th2 paradigm of immune regulation, and its relationship to immune-mediated human diseases has become increasingly accepted in recent years. Stated briefly, polarised Th1 cells secrete immune activating cytokines such as interferon-γ, (IFN-γ) and promote cell-mediated immune responses (eg killing of intracellular parasites). Polarised Th2 cells secrete cytokines such as interleukin-4 (IL-4), IL-5 and IL-10. Th1 and Th2 cytokines have a tendency for mutual inhibition, potentially creating conditions under which subtle disturbances can lead to pathological imbalance over a period of time. An imbalance of Th2 over Th1 immunity is associated with allergic disease and hypersensitivity (reviewed in Abbas et al, 1996) and mood changes including depression (reviewed in Rook & Zumla, 1997). These observations form the basis of the proposal by Rook and Zumla that such an imbalance could be associated with Gulf War-related illness. In recent years it has become possible to measure the products (cytokines) of Th1 and Th2 lymphocytes in peripheral blood with great sensitivity and accuracy. In some disorders, such measurements correlate very closely with the proposed immune pathogenesis of the disease. In allergy, for example, a preponderance of Th2 activity is observed in peripheral blood (Romagnani, 1994), consistent with the fact that Th2 cytokines promote the production of allergen-specific IgE and eosinophil recruitment, the two main immunopathological hallmarks of clinical allergy.

There is no single bio-assay to establish the Th1/Th2 balance in an individual. The current "state of the art" suggests that a multi-layered approach should be employed initially, to identify a set or sets of examinations which provide meaningful data. In our Statement of Work we proposed to begin with an examination of the intracellular production of cytokines within mitogenically stimulated CD4 and CD8 T cells. The establishment and validation of this technique, and the results from this study form the major part of this report.

Body

Patients

The blood samples in this study were drawn from a subset of veterans in the previously reported large epidemiological study (Unwin et al, 1999). These stage 2 samples were randomly selected from veterans who participated in the stage 1 study. We have selected at random a sample of sick Gulf veterans, defined as all those veterans who scored 72.2 or less on the SF-36 physical functioning subscale. This was the cut-off value for the lowest 10th centile of the distribution of the SF 36 PF in the Era (baseline group). There are two control groups. Similar numbers of sick veterans of the Bosnia conflict or sick service personnel who were not deployed in either conflict (era controls) and fulfill the same criteria for illness have been recruited, as have similar numbers of well Gulf veterans. Tests carried out in conjunction with sampling, and which form the basis of the stage 2 study, include clinical assessments, psychiatric and neuropsychiatric screening, respiratory function, and also in a sub group intensive neurophysiological and neuromuscular investigations. All subjects are also asked to bring their vaccination records with them where available, and to consent to medical record review. In addition, we are currently acquiring medical records in order to obtain definitive information regarding vaccine status for each participant. It should be noted that at the time of preparation of this report, data on clinical assessments, medical record review and vaccine records are not yet available.

Blood samples, drawn from epidemiologically defined cohorts and healthy laboratory controls, were processed and analysed by an individual lab worker blinded to the source of the sample. The Gulf veterans in the study had seen service in the UK military in the Gulf region between September 1, 1990 and June 30, 1991. We studied 54 patients who are without any symptoms of the "Gulf War related illness" (Gulf well veterans - GWV) and 60 veterans who are ill. As a first matching control group were 18 randomly chosen veterans who had served in Bosnia between April 1, 1992, and February 6, 1997 and 37 personnel serving in the armed forces on January 1, 1991, who were not deployed to the Gulf War (Era cohort). The Era cohort control group was matched for sex, age, rank, and fitness service status, while the Bosnia control group was matched only for sex, age, rank because the service status and fitness data were not available. As a third control group we used 24 hospital/laboratory professionals matching for sex and age.

Methods adopted for determination of intracellular cytokine secretion

Heparinised whole venous blood was used for the in vitro cell stimulation, in order to observe the whole potential of cytokine production by lymphocytes. This method avoids partial activation of lymphocytes arising from the Ficoll-Hypaque gradient separation procedure. The whole blood culture prevents the unintentional separation of neighboring cells and serum components so that they retain their in vivo characteristics. Cytokine production in whole blood systems has also been reported to be greater than in purified PBMC preparations (De Groote et al, 1992).

We evaluated in an initial pilot study several protocols for the measurement of intracellular cytokine staining based on published papers. These included the

assessment of various combinations of: mitogens (phorbol myristate acetate (PMA), phytohaemaglutinin (PHA), ionomycin, anti-CD3, and anti-CD28). We also examined which concentration of the various mitogens offered the greatest sensitivity over background (signal/noise ratio). As a result we chose to use in the main study the polyclonal stimulants PMA and ionomycin as mitogens. These gave the best yield of cytokine production by lymphocytes compared to the other stimulants investigated. In previous studies PMA and ionomycin were also shown to be superior (Saner et al, 1996; North et al, 1996). In the study of intracellular cytokine expression, blockers of protein secretion are added in order to retain cytokines within the cell. We investigated the protein secretion inhibitors monensin and Brefeldin A added either immediately or 1-2 hours after stimulation. Monensin is an inhibitor of trans-Golgi function, while Brefeldin A inhibits protein transport between the endoplasmic reticulum and the Golgi apparatus. In the literature these two inhibitors used alone have produced inconsistent results (Jung et al, 1993; Prussin & Metcalfe, 1995; Nylander & Kalies, 1999). We adopted their use in combination, added immediately for the main study as this proved to provide the most effective yield of cytokines. Finally, we investigated in pilot trials the length of culture (6, 16, 24 hours). It is well known that each cytokine has different kinetics of intracellular expression so that they reach their respective maxima at different time points. The literature is inconclusive, with optimal values varying from 4 hours for IL-4 up to 64 hours for IFN-γ. In our own pilot evaluation we found that a 16-hour incubation period gave effective results for all of the 4 cytokines we were investigating.
In the main study therefore we cultured whole blood for 16 hours in the presence of Brefeldin A, monensin and the polyclonal activators PMA and ionomycin, followed by staining of CD4+ and CD8+ cells for IL-4, IFN-γ, IL-2, IL-10.

Heparinised whole blood was mixed with culture medium (RPMI1640 + 10%FCS + 2mM L-glutamine + 100IU/ml penicillin + 100μg/ml streptomycin) in proportion: 1 part blood to 2.6 parts of culture medium. The mixture was placed in 1ml aliquots into separate wells on a 24-well TC plate. One set of the wells was then supplemented with 5ng/ml of PMA and 745ng/ml of ionomycin. Protein secretion inhibitors Brefeldin A (5μg/ml) and monensin (2.08mg/ml) were added and blood cultured at 37°C for 16 hours in an atmosphere of 95% air and 5% CO_2. As a negative control we also measured the intracellular cytokine production from a duplicate set of cultures in wells containing resting cells. These contained no polyclonal stimulators and were incubated with the protein blockers Brefeldin A and monensin only.
The cells were then harvested, washed and stained with PerCP conjugated anti-human CD4 monoclonal Antibodies (mAb) and FITC-conjugated anti-human CD8 (both from Becton Dickinson) in order to phenotype the cells of interest. Then the cells were washed 2 times with PBS containing 5% FCS. The intracellular cytokine staining protocol was followed as suggested by the manufacturer (Caltag Lab) of the Fix&Perm Permeablilization Kit used in our studies. In brief, cells were fixed with Reagent A (Fix&Perm Permeablilization Kit) and incubated for 15 minutes at room temperature in the dark. After washing 2 times the Reagent B was added (as a permeabilization solution) along with appropriate PE-conjugated anti-cytokine (IL-2, IFNγ, IL-4, IL-10) mAbs or fluorochrome and isotype-matched mAbs as controls (all from Pharmingen)

and incubated at 4°C for 30 minutes. Finally, the stained cells were washed and suspended in 200μl of PBS for flow cytometry analysis.

Intracellular cytokine production by CD4 and CD8 cells was assessed. Ten thousand events of gated lymphocytes (on forward and side scatter) were acquired and analyzed using CELLQuest software (Becton Dickinson). Dot plot quadrant statistics were set on the basis of corresponding isotype-matched control mAb during data analysis such that the frequencies of CD4 or CD8 positive cell populations capable of IL-2, IFN-γ, IL-4, IL-10 production were determined.

Results

The results are shown in tables1-4 and figures 1 and 2 in the appendices, pages 15-20. In interpreting the results, we take the view that cells which demonstrate the presence of intracellular cytokines without stimulation represent a *recently activated* population. Cells which require mitogenic stimulation to reveal cytokine secretion represent *resting memory* cells. These two populations are considered separately.

CD4 T cell interleukin-4 secretion. Resting (unstimulated) IL-4 secretion is typically present at very low levels, as can be seen in the laboratory healthy controls. Similar low levels were seen in the sick Bosnia/Era cohort and in the well GWVs. However, significantly elevated levels of resting IL-4 secreting CD4 T cells were seen in sick GWVs compared with laboratory controls ($p=0.0019$) and the other service groups ($p=0.0002$ and 0.0007).
Following mitogenic activation, no increase was seen in IL-4 secreting cells in healthy laboratory controls. This reflects the fact that in health, circulating activated IL-4 secreting cells are rarely seen. In contrast, levels of IL-4 secreting CD4 cells were significantly elevated in all veteran groups compared with controls ($p<0.0001$ for all) and were higher in sick GWVs than in the other veteran groups ($p=0.0129$ and 0.0091).

CD4 T cell interferon-γ secretion. Resting (unstimulated) IFN-γ secretion followed a similar pattern to IL-4, in that levels were only significantly raised in sick GWVs compared with controls ($p=0.0014$), sick Bosnia/Era cohort ($p=0.0129$) and well GWVs ($p=0.0091$). After stimulation, there was a rise in levels of IFN-γ secreting cells in all of the study groups and controls, such that levels were similar in each group.

CD4 T cell interleukin-2 secretion. Again, resting (unstimulated) IL-2 secretion followed a similar pattern to IL-4 and IFN-γ, in that levels were only elevated in sick GWVs compared with controls ($p=0.0093$), Bosnia/Era cohort ($p=0.0017$) and well GWVs ($p=0.0054$). After activation, levels rose in all groups, such that levels were similar in each group.

CD4 T cell interleukin-10 secretion. Resting IL-10 secretion was significantly elevated in all study groups compared with laboratory controls. Levels rose in all study groups after stimulation, and were significantly higher in all veteran groups than in laboratory

controls. A distinct pattern of IL-10 expression emerged, in that levels were higher in sick veterans (both sick Bosnia/Era and sick GWVs) compared with well GWVs ($p=0.01$ and 0.004, respectively).

Changes in intracellular cytokine expression by CD8 cells were similar to those seen in CD4 cells, but had a less distinct pattern (figure 2).

Key Research Accomplishments

- We have examined intracellular Th1 and Th2 cytokine expression in a large cohort of patients from the following groups: Sick GWVs, well GWVs and sick Bosnia/Era veterans.

- Sick GWVs are characterised by the presence of significantly expanded populations of CD4 cells which, without stimulation, secrete IL-4, IFN-γ, IL-2 and IL-10. This is the so-called Th0 pattern, and these cells are likely to be recently activated.

- In contrast, after stimulation, sick GWVs demonstrate high levels of memory cells which secrete IL-4. Additional analyses (data not shown) have confirmed that these cells do not express IFN-γ and are therefore true Th2 cells.

- Secretion of IL-10 (considered a Th2 or Th3 (Tr1) cytokine) by memory cells is elevated in all study groups, but most markedly in sick veterans, irrespective of deployment.

In summary, these results demonstrate a complex pattern of abnormalities. Sickness amongst GWVs is associated with ongoing Th0 immune activation and evidence of previous Th2 polarisation amongst memory CD4 cells. Sickness typical of GW deployment is associated with induction of memory CD4 cells secreting the regulatory cytokine IL-10.

A critical component of this study that remains to be factored into the analysis is the vaccine history. At the time of writing the report, vaccine data were still too scanty to be amenable to analysis.

Reportable Outcomes

In view of the lack of disease and vaccine correlates, we consider these data too preliminary to have reported them in the literature or at scientific meetings.

Conclusions

Our results demonstrate unusual but consistent changes in cytokine secretion in GWVs. Certainly it can be concluded that their cytokine profiles are not normal. Of particular interest is the Th2 shift in sick GWVs. Addition of data on vaccine exposure will enable us to examine whether this Th2 shift is associated with vaccine exposure. However, since a Th2 shift was also seen in Bosnia/Era controls, multiple vaccines cannot be the only immunological stimulus capable of giving this outcome.

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Table 1 Expression of intracellular cytokine production by CD4+ cells within lymphocyte population in all cohorts

	% IFN - γ		% IL - 4		% IL - 2		% IL - 10	
	Resting	Activated	Resting	Activated	Resting	Activated	Resting	Activated
Lab controls	0.92 (0.73)	13.28 (3.93)	1.95 (0.94)	1.87 (0.89)	0.84 (0.66)	18.64 (4.93)	0.83 (0.45)	2.32 (1.01)
BI+EI	1.28 (1.00)	11.88 (4.12)	2.06 (0.88)	3.58 (1.88)	0.97 (0.83)	16.81 (6.28)	1.56 (1.12)	4.14 (1.50)
GW	1.30 (1.02)	11.79 (4.58)	2.10 (0.99)	3.60 (1.91)	1.02 (0.89)	16.91 (6.94)	1.57 (1.06)	3.25 (1.57)
GI	1.72 (1.19)	11.69 (5.01)	3.03 (1.51)	4.50 (2.16)	1.46 (1.00)	17.07 (5.47)	1.71 (1.03)	4.43 (1.85)

Mean (SD) percentage of total lymphocytes positive for different cytokines and CD4 under resting (ie unstimulated) and mitogen-activated conditions. BI, ill Bosnia veterans; EI, ill era controls; GW, well Gulf veterans; GI, ill Gulf veterans.

Table 2 Expression of intracellular cytokine production by CD8+ cells within lymphocyte population in all cohorts

	% IFN - γ		% IL - 4		% IL - 2		% IL - 10	
	Resting	Activated	Resting	Activated	Resting	Activated	Resting	Activated
Lab controls	0.50 (0.41)	11.86 (4.87)	1.21 (0.68)	0.87 (0.68)	0.47 (0.39)	3.00 (1.79)	N/A	N/A
BI+EI	0.91 (0.80)	9.37 (5.08)	1.26 (0.79)	1.01 (0.78)	0.60 (0.58)	2.93 (1.69)	1.04 (0.73)	1.48 (0.96)
GW	1.21 (0.91)	8.72 (3.92)	1.55 (0.99)	1.18 (0.75)	0.95 (0.80)	2.83 (1.73)	1.44 (1.13)	1.24 (0.78)
GI	1.19 (0.94)	8.73 (3.76)	1.69 (0.93)	1.48 (0.96)	0.79 (0.65)	2.83 (1.58)	1.13 (0.96)	1.41 (1.06)

Mean (SD) percentage of total lymphocytes positive for different cytokines and CD8 under resting (ie unstimulated) and mitogen-activated conditions. BI, ill Bosnia veterans; EI, ill era controls; GW, well Gulf veterans; GI, ill Gulf veterans.

Table 3 P values calculated by Mann – Whitney test between cohorts for CD4+ cells

Comparison of cohorts	IL-4		IFN-γ		IL-2		IL-10	
	Resting	Activated	Resting	Activated	Resting	Activated	Resting	Activated
Lab controls vs BI+EI	0.5872	**<0.0001**	0.1428	0.1195	0.5965	0.1026	**0.0238**	**<0.0001**
Lab controls vs GW	0.5590	**<0.0001**	0.1990	0.0908	0.5303	0.2766	**0.0065**	**0.0224**
Lab controls vs GI	**0.0019**	**<0.0001**	**0.0014**	0.0960	**0.0093**	0.2119	**0.0005**	**<0.0001**
BI+EI vs GW	0.8784	0.7504	0.8906	0.8154	0.6645	0.9452	0.88	**0.01**
BI+EI vs GI	**0.0002**	**0.0129**	**0.0245**	0.5282	**0.0017**	0.7445	0.47	0.44
GW vs GI	**0.0007**	**0.0091**	**0.0359**	0.7899	**0.0054**	0.8590	0.49	**0.004**

Statistically significant differences are given in bold. BI, ill Bosnia veterans; EI, ill era controls; GW, well Gulf veterans; GI, ill Gulf veterans.

Table 4 P values calculated by Mann – Whitney test between cohorts and lab control in CD8+ cells

Comparison of cohorts	IL-4		IFN-γ		IL-2		IL-10	
	Resting	Activated	Resting	Activated	Resting	Activated	Resting	Activated
Lab controls vs BI+EI	0.9201	0.3711	0.0081*	0.0086*	0.3944	0.9704		
Lab controls vs GW	0.2369	0.0927	0.0012*	0.0309*	0.0166*	0.5932		
Lab controls vs GI	0.0434*	0.0072*	0.0002*	0.0069*	0.0372*	0.9617		
BI+EI vs GW	0.1427	0.1855	0.0930	0.7307	0.0179*	0.6044		
BI+EI vs GI	0.0115*	0.0041*	0.0522	0.8548	0.0533	0.9304		
GW vs GI	0.3250	0.1552	0.8250	0.8995	0.4302	0.5893		

Statistically significant differences are given in bold. BI, ill Bosnia veterans; EI, ill era controls; GW, well Gulf veterans; GI, ill Gulf veterans.

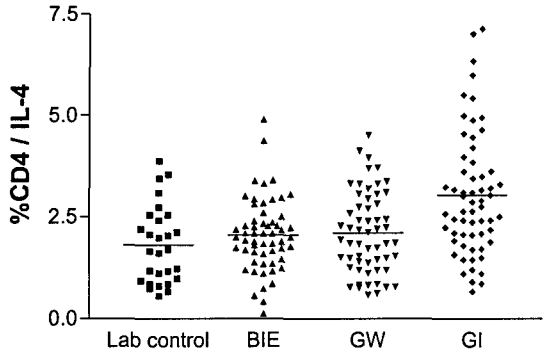
Appendix V
Figure 1

UNSTIMULATED

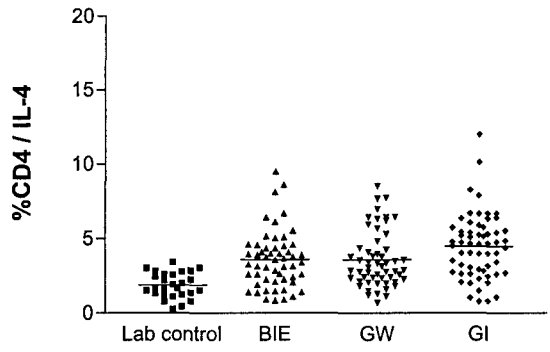
ACTIVATED

UNPUBLISHED DATA

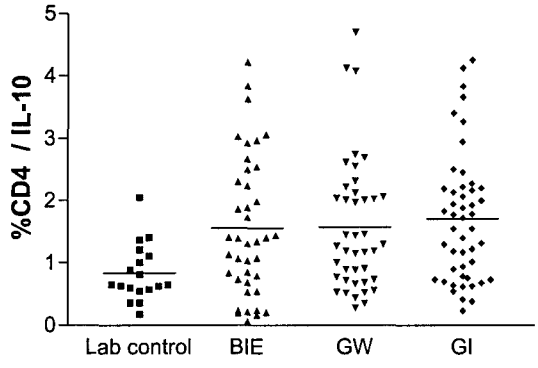
CD4+ / IL-4 unstimulated



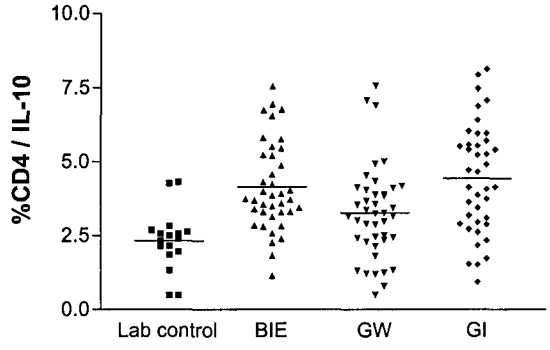
CD4+ / IL-4 activated



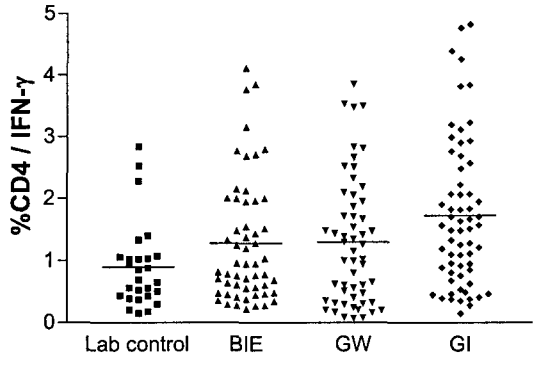
CD4+ / IL-10 unstimulated



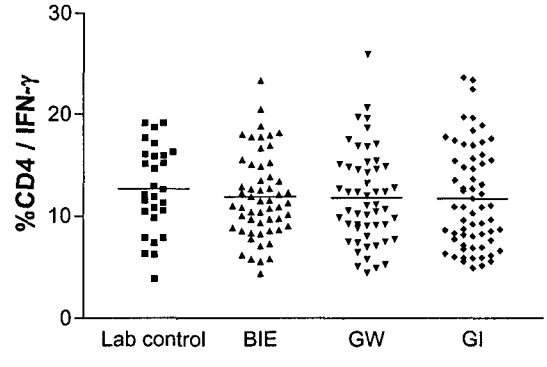
CD4+ / IL-10 activated



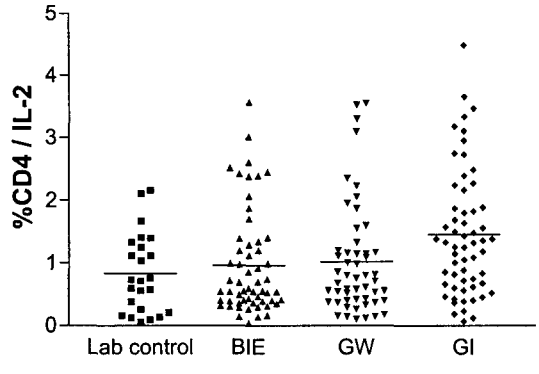
CD4+ / IFN-γ unstimulated



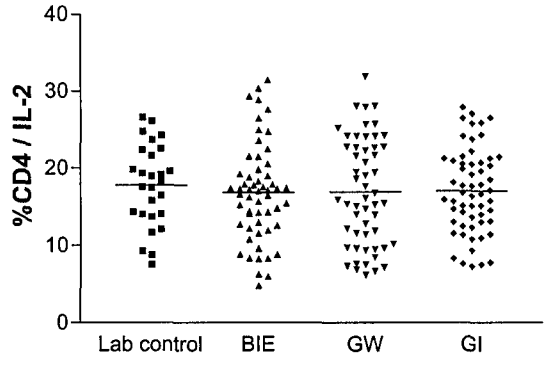
CD4+ / IFN-γ activated



CD4+ / IL-2 unstimulated

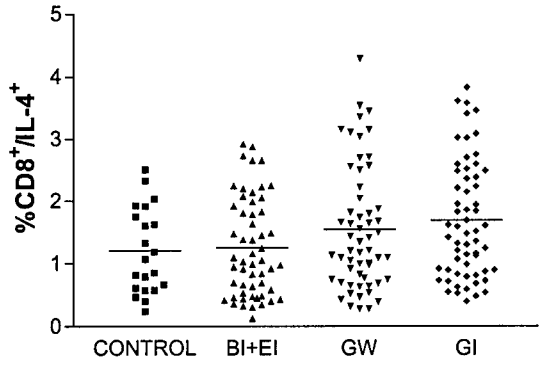


CD4+ / IL-2 activated

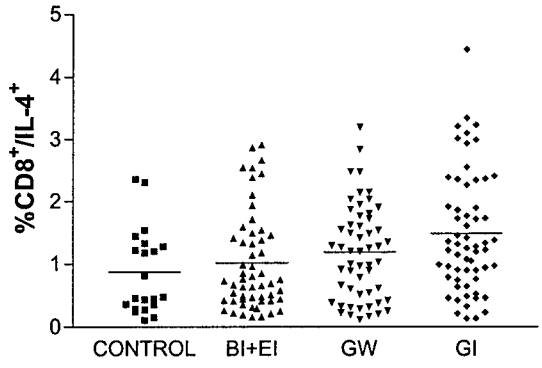


Appendix VI Figure 2

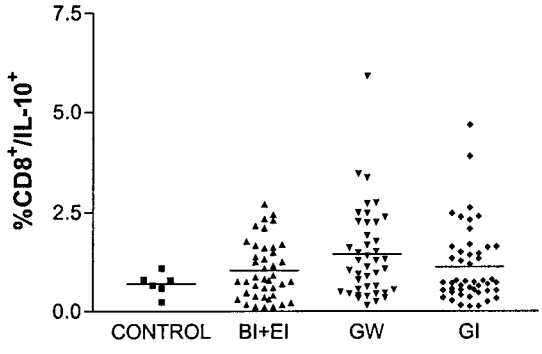
CD8/lymph/IL-4 - unstimulated



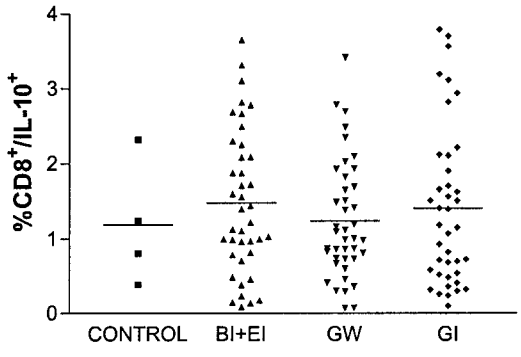
CD8/lymph/IL-4 - stimulated



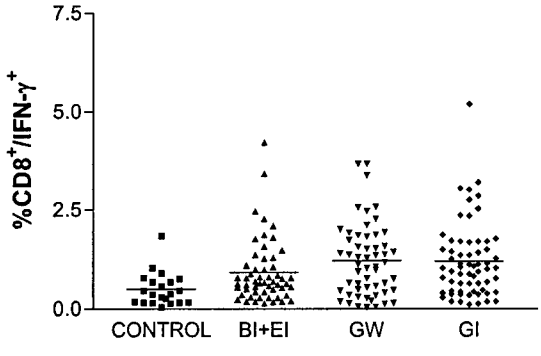
CD8/lymph/IL-10 - unstimulated



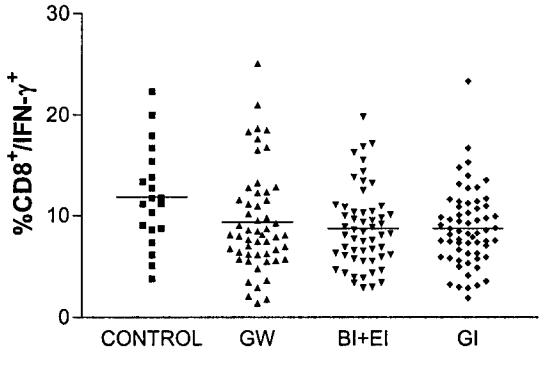
CD8/lymph/IL-10- stimulated



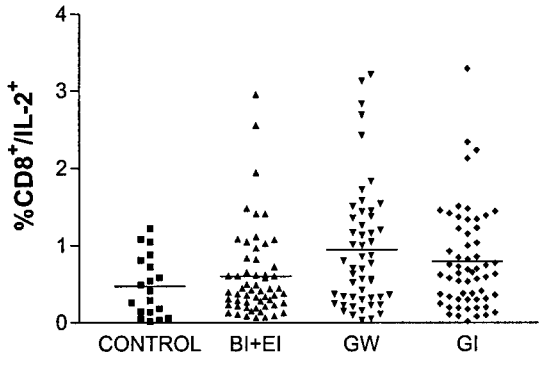
CD8/lymph/IFN-γ - unstimulated



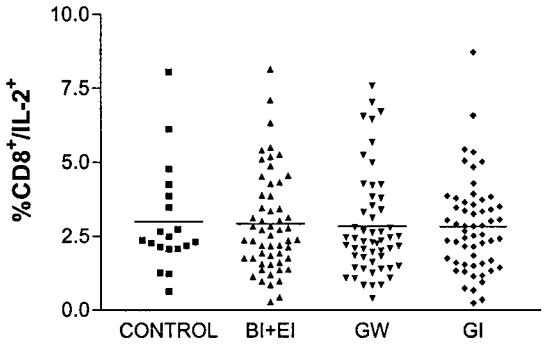
CD8/lymph/IFN-γ - stimulated



CD8/lymph/IL-2 - unstimulated



CD8/lymph/IL-2 - stimulated



Appendix VII

Legends to figures

Figure 1
Percentage cytokine positive CD4+ T cells identified by intracellular cytokine staining (IL-4, IL-10, IFN-γ and IL-2) of whole peripheral blood. Panels on the left are from cultures left unstimulated and on the right from cultures activated with PMA and ionomycin. Horizontal bars represent means. For statistical comparisons, see Tables 1-4, Appendices I-IV. Values are given for healthy laboratory controls, sick veterans of the Bosnia campaign/non-deployed service staff (Era controls; BI+EI), well veterans of the Gulf War (GW) and ill veterans of the Gulf War (GI).

Figure 2
Percentage cytokine positive CD8+ T cells identified by intracellular cytokine staining (IL-4, IL-10, IFN-γ and IL-2) of whole peripheral blood. Panels on the left are from cultures left unstimulated and on the right from cultures activated with PMA and ionomycin. Horizontal bars represent means. For statistical comparisons, see Tables 1-4, Appendices I-IV. Values are given for healthy laboratory controls, sick veterans of the Bosnia campaign/non-deployed service staff (Era controls; BI+EI), well veterans of the Gulf War (GW) and ill veterans of the Gulf War (GI).

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 8 Jan 2003

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

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1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to the enclosed. Request the limited distribution statement for the enclosed be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

PHYLLIS M. RINEHART
Deputy Chief of Staff for
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