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Cellular Immune Activation in Gulf War Veterans

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The etiology and pathology of illnesses related to the first Persian Gulf War are unclear. Among the constellation of symptoms noted in sick veterans, some, such as skin rashes, musculoskeletal pains, and neuropsychiatric problems, have been proposed to reflect an underlying immune dysfunction. In this study we explored the hypothesis that sickness following deployment to the Gulf in 1991 is associated with altered immune function, and we examine possible associated exposures. In particular, we focused on peripheral blood Th1/Th2 balance by measuring intracellular production of IFN-γ, IL-2 (Th1), IL-4 (Th2), and IL-10 by CD4 T cells, using a nested case control study design within a large epidemiological survey. We compared symptomatic Gulf War veterans (sGWV) with well GWVs (wGWV), and a second control group of symptomatic veterans who served in Bosnia or were nondeployed military personnel of the same era. We found evidence for an altered immune status in sGWV in comparison to the other study groups. In particular, ongoing Th1-type immune activation was associated with multisymptom illness in GWVs, with sick veterans having significantly elevated levels of IFN-γ and IL-2 producing CD4+ cells in the absence of *in vitro* stimulation compared with wGWVs ($P = 0.01$ and $P = 0.001$). *In vitro* polyclonal activation revealed significantly elevated levels of IL-10 producing memory CD4 cells in sGWVs ($P < 0.001$), but other cytokines were normal. In terms of possible exposures that might influence immune function, we found a trend for reduced levels of IFN-γ producing cells after polyclonal activation with increasing numbers of vaccines administered ($P < 0.05$) but no changes in other cytokines. These data show that multisymptom illness in Gulf War veterans is characterized by ongoing Th1-type immune activation and a biased generation of memory cells secreting the suppressor cytokine, IL-10.

KEY WORDS: Gulf War; cytokines; Th1/Th2 balance; Gulf War-related illness.

INTRODUCTION

Almost 53,000 United Kingdom service personnel took part in the 1990–1991 Persian Gulf War. Following their return, many Gulf War veterans from the United Kingdom and United States sought medical advice for symptoms they felt were related to their wartime service. Our previous large epidemiological study showed that Gulf War veterans had markedly increased rates of ill health compared with nondeployed service personnel and veterans of peacekeeping duties in Bosnia. Our study and others have failed to find any evidence of a discrete specific syndrome (1–4), although other studies have reported such a finding (5, 6). The most frequent complaints among veterans are of fatigue, rashes, joint and/or muscle pain, neuropsychiatric complaints, shortness of breath, sleep disturbances, and gastrointestinal problems (2). Various etiologies have been proposed, including post-traumatic stress disorder (7), possible lead absorption from oil-well fire smoke, the diesel stoves used to heat tents, and depleted uranium (8), anti-nerve gas drugs (9), chemical weapons (10), insect repellents (11), and multiple vaccinations (12).

A popular hypothesis, proposed by Rook and Zumla, argued that Gulf War illnesses were the result of a T helper 2 (Th2)-biased immune response (13). The T helper1/Thelper2 (Th1/Th2) paradigm of immune responsiveness has been used to further the understanding of a number of disease states associated with immune dysfunction, such as allergy (14) and autoimmune disease (15). Th2-associated disorders such as hypersensitivity (14) and mood changes (16) are similar to symptoms reported by some veterans. Rook and Zumla noted that Gulf War

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veterans were exposed to several stimuli that strongly favor a Th2-biased immune response. First, biological warfare vaccines were given under stressful, near-combat conditions during deployment. Stress-associated glucocorticoid hormones deviate the immune response toward Th2 under experimental conditions (17–20). Second, although natural infection with *Bordetella pertussis* and its whole cell-derived vaccine promotes a strong Th1 response, paradoxically the acellular vaccine component of pertussis toxin used as an adjuvant by U.K. forces in the Gulf can cause Th2 deviation (21, 22).

Recently, the Rook and Zumla hypothesis received some support from an extension of our epidemiological study, showing that receiving vaccinations after deployment to the Gulf, and receiving multiple vaccinations, were associated with multisymptom illness in the Gulf War veteran cohort (12). To explore the link between cytokine imbalance and illness, we studied cytokine profiles in Gulf War veterans with and without multisymptom illness and with a range of vaccine exposures. To examine whether cytokine imbalance was peculiar to Gulf service, we also studied a cohort of nondeployed service personnel and veterans of peacekeeping duties in Bosnia who displayed similar multisymptom ill health.

METHODS

Patients and Study Design

Blood samples were obtained from volunteers attending the Gulf War Illness Research Unit at GKT Medical School who were participants in our cross-sectional Stage II analysis, following on from the questionnaire based Stage I epidemiological study previously reported (2). Stage I was a random sample of veterans of the Gulf conflict (1990–1991) ($n = 5046$), veterans of the Bosnia peace keeping mission (1992–997) ($n = 3450$), and veterans in active service 1990–1991 who were not deployed to the Gulf ("Era" veterans) ($n = 4248$). These veterans were sent postal questionnaires, and the details of recruitment, tracing, response rates, and baseline levels of ill health in Stage I have been reported elsewhere (2). In Stage II, four samples were randomly selected based on health status measured at Stage I. Ill health was defined as impaired physical functioning using the Short Form 36 Physical Functioning measure (SF-36PF; see below) (23). The numbers of symptomatic Gulf, nonsymptomatic Gulf, symptomatic Bosnia, and Era veterans were 406, 3047, 138, and 278, respectively, from which random samples were invited to attend the Gulf War Illnesses research unit at King's College London for a standardized clinical evaluation, between January 1999 and September 2000. The study had ethical committee approval and informed consent was obtained from each subject. Overall, 740 cohort members were eligible for the study. Contact was made with 607. 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 participation rate from Stage I to Stage II was 67% ($n = 111$) for symptomatic Gulf veterans, 62% ($n = 98$) for well Gulf veterans, 55% ($n = 54$) for symptomatic Bosnia veterans, and 43% ($n = 79$) for symptomatic Era veterans (χ^2 test for heterogeneity $= 28.0$, df $= 3$, $P < 0.0005$).

Acquisition for the current study commenced January 1999 and was continuous to June 1999 and between September 1999 and September 2000. Of those who attended Stage II of the study (total $n = 342$), we were able to obtain sufficient blood for immune analysis from 80 of 98 (82%) symptom-free veterans (well Gulf War veterans; wGWV) and 40 of 111 (36%) veterans who were symptomatic (sGWV). To examine effects specifically associated with Gulf War-related illness the study also included 58 military personnel, either veterans of peacekeeping duties in Bosnia ($n = 20$) or in military service in 1991 but not actually deployed to the Gulf War ($n = 39$; total, 59/133 [44%]; χ^2 test for heterogeneity $= 6.3$, df $= 2$, $P = 0.04$) who also displayed multisymptom illness (symptomatic Bosnia/Era control group, sBEV).

Illness status was assigned according to the Stage II analysis. In the absence of an accepted or meaningful classification of Gulf War-related multisymptom illness, we chose a case definition based on symptomatic and functional ill health. Symptomatic individuals (sGWV and sBEV) were defined as those who had scored 72.2 or less on the SF-36 Physical Functioning subscale. This was the cutoff value for the lowest 10th centile of the distribution of the SF-36 PF in the Stage I analysis of era personnel. The study was performed blindly using coded blood samples from the four defined groups and was approved by the Institute's Ethical Review Committee (LREC 96-172a). As depression was more common in the sGWV group and has been associated with a range of immune changes, we measured depression on the Beck Depression Inventory (BDI) when participants attended for their detailed medical assessment.

Vaccine Exposure

Exposure to vaccines was recorded in two ways. First, with the individual's consent, we attempted to trace personal medical records for study participants to gain details of recorded exposure to vaccines in the period June 1990 to February 1991. From this we were able to calculate

the total vaccines received. For the purposes of studying associations between multiple vaccines and cytokines, individuals were assigned to one of five quintile groups on the basis of number of vaccines received, as described previously (and see below) (12). Second, respondents to the original survey completed a questionnaire which included details about (1) whether the serviceman had his vaccine record, (2) how many and which vaccines he received in the two months prior to deployment, and (3) how many and which vaccines he received during deployment. From the replies to these questions we calculated the total number of vaccines received. We assumed that the vaccine records would be more accurate than reported vaccines, so in individuals with vaccine records (95/120 GWV), records were used. For those without records, reported vaccine exposure was used.

Cell Culture and Intracellular Cytokine Staining

Flow cytometry was used to measure intracellular cytokine production by CD4 T lymphocytes. Heparinized venous whole blood was 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 [FCS], 2 m*M* L-glutamine, 100 IU/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 a 5 ng/ml final concentration of M phorbol 12-myristate 13-acetate (PMA) and 745 ng/ml 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 hr 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 (PBS) containing 5% FCS and 0.01% sodium azide, and intracellular cytokine staining carried out 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 min at room temperature in the dark. After washing twice, permeabilizing Reagent B was added along with appropriate PE-conjugated anticytokine (IL-2, 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 min. Finally, the stained cells were washed and suspended in 200 μl of PBS containing 0.01% sodium azide for flow cytometry analysis. Additional studies were carried out 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 are expressed as percentage positive of total lymphocytes.

Statistical Analysis

We determined that all the cytokine data were normally distributed and, therefore, used *t*-tests to assess differences between the sGWV and the wGWV. Duplicate analyses performed using nonparametric statistical tests gave similar results. We used multiple regression analysis to determine mean differences and 95% confidence intervals for cytokine levels between the two groups. We controlled for age, gender, vaccination status, antidepressant use, mood (BDI score), and history of atopic illnesses. Results are expressed as mean differences between groups with 95% confidence intervals.

RESULTS

Immune Activation in Nonstimulated CD4 Cells

CD4+ lymphocytes cultured in the absence of polyclonal activators were examined for intracellular expression of IFN-γ, IL-2, IL-4, and IL-10. Cytokine-positive nonstimulated CD4+ cells most likely represent those lymphocytes already activated *in vivo* when blood was drawn. Although there was overlap between groups, we found evidence of ongoing immune activation in the sGWV group, compared with the wGWV and sBEV groups (Fig. 1). GWVs defined as symptomatic had significantly higher mean levels of nonstimulated, IL-4+ and IL-2+ cells compared with well GWVs and symptomatic BEVs (for IL-4, sGWV compared to wGWV, $P < 0.05$, and sGWV versus sBEV, $P < 0.001$; for IL-2, for sGWV versus wGWV, $P < 0.01$, and for sGWV versus sBEV, $P < 0.001$). Levels of IFN-γ producing cells in sGWV incubated without polyclonal activator were higher in comparison to wGWV, though not significantly so, and significantly higher in comparison to the sBEV

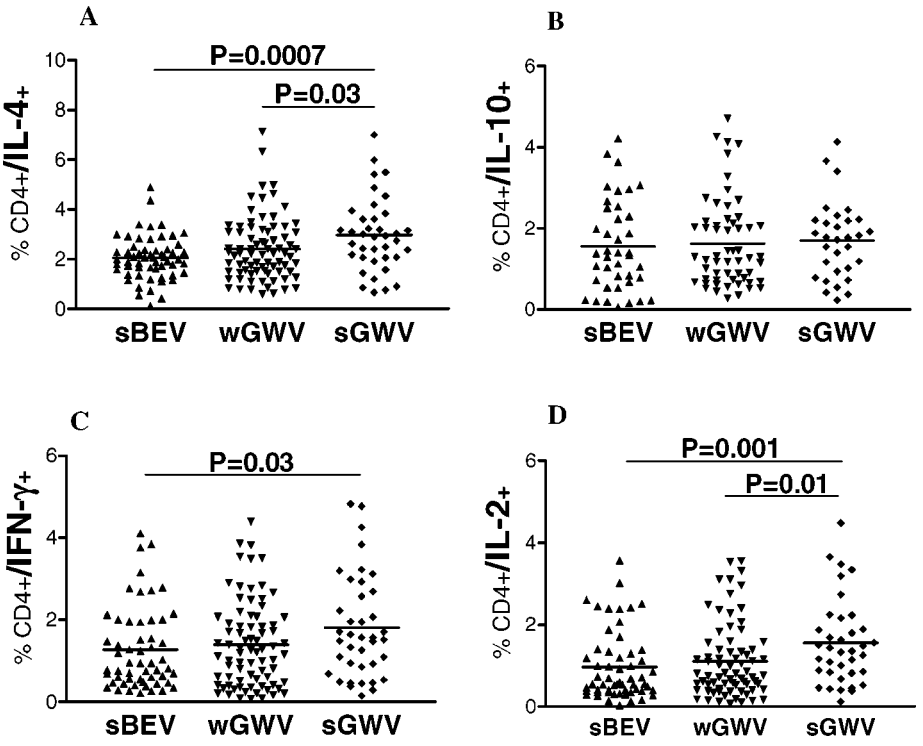

Fig. 1. Evidence for ongoing Th0 immune activation in Gulf War veterans with multisymptom illness. Graph shows percentage of nonstimulated CD4+ T cells staining for Th2 (A; IL-4+ cells), Th1 (C and D, IFN-γ+ and IL-2+ cells, respectively), and Tr (B; IL-10+ cells) in Bosnia/Era veterans with multisymptom illness (sBEV) and Gulf War veterans with and without multisymptom illness (sGWV and wGWV, respectively).

control group ($P < 0.05$). Levels of IL-10 secreting cells in nonstimulated cultures were similar in all groups. This profile suggests a mixed, Th0-type pattern of ongoing CD4 T cell activation.

Memory Cell Cytokine Balance in Gulf War Veterans

After short-term polyclonal stimulation *in vitro*, memory cells produce cytokines that reflect their effector polarization, whereas naïve cells do not respond under these conditions. In such polyclonally stimulated cells, we found no evidence of an altered Th1/Th2 immunity in symptomatic GWVs. Levels of IL-4, IFN-γ, and IL-2 producing CD4 cells were similar in all study groups (Fig. 2). However, we also measured levels of IL-10 producing cells, since this cytokine is secreted by Th2-like cells (24) and regulatory T cells (Tr) and has also been reported to be increased in patients with depression (25), a clinical feature of some of our veterans (2). Interestingly, in GWVs the mean level of IL-10 producing CD4 cells was significantly higher in symptomatic GWVs than in wGWVs ($P < 0.0001$) and symptomatic BEVs ($P = 0.05$) (Fig. 2). Levels were also higher in sBEVs than in wGWVs, suggesting that elevated levels of IL-10 secreting cells are associated with both illness and Gulf deployment.

Illness Effects on Cytokine Balance in Gulf War Veterans

Our data suggest that multisymptom illness among GWVs, reported through our Stage II questionnaire-based analysis, is associated with ongoing immune activation (seen in nonstimulated cells) and with elevated level of regulatory/suppressor IL-10-producing T cells after polyclonal stimulation. To examine the effects of illness on these markers, we compared results between symptomatic and well GWVs using a multiple regression model. No single symptom was significantly associated with any single immune parameter we measured.

Table I compares levels of nonstimulated cytokine-positive cells in sGWVs compared with wGWVs. sGWVs had higher levels of IL-4, IFN-γ, and IL-2 compared with wGWVs. After controlling for age, gender, vaccination status, antidepressant use, depressed mood, and history of atopic illness, the association with IL-4 was lost but remained significant for IFN-γ and IL-2.

Table II shows the results for polyclonally activated cytokine-positive CD4 cells according to illness status.

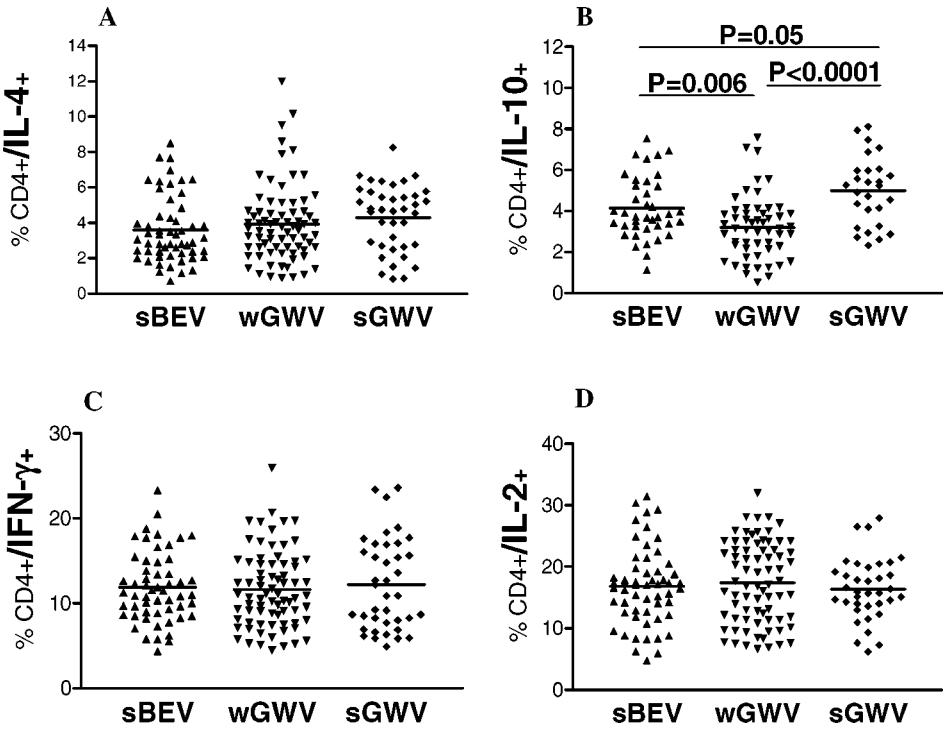

Fig. 2. Increased production of regulatory IL-10 in Gulf War veterans with multisymptom illness. Graph shows percentage of polyclonally activated CD4 T cells staining for Th2 (A; IL-4+ cells), Th1 (C and D, IFN-γ+ and IL-2+ cells, respectively), and Tr (B; IL-10+ cells) in Bosnia/Era veterans with multisymptom illness (sBEV) and Gulf War veterans with and without multisymptom illness (sGWV and wGWV, respectively).

These show that sGWVs had higher levels of IL-10+ cells. Controlling for clinical variables (for age, gender, vaccination status, antidepressant use, depressed mood, and history of atopic illness), this immune change remained significant.

Taken together, these results suggest that the cardinal immunological features of multisymptom illness in GWVs are ongoing immune activation which is predominantly Th1 and, more notably, expansion of IL-10 producing memory cells.

Vaccine Effects on Cytokine Balance in Gulf War Veterans

Our previous research indicated a weak association between multiple vaccines and symptoms but not disability measured on the SF-36 Physical Functioning scale. Therefore it may not be surprising that vaccine status in sGWVs and wGWVs did not differ in the present study. We assessed the association between multiple vaccines and physical symptoms in the sample for whom we had immune data and were unable to find an association between ill health and multiple vaccines in this sample. This

Table I. Relationship Between Gulf Illness Status as Defined from Stage II and Levels of Nonstimulated Cytokine Producing CD4+ Cells

T cells	Symptomatic GWVs (%)	Well GWVs (%)	Mean difference (95% CI) corrected for: Age and gender	Mean difference (95% CI) corrected for: Age, gender, vaccination status, antidepressant use, BDI score, and history of atopic illness
Th2				
IL-4	2.98	2.40	0.57 (0.03, 1.11), $P = 0.04$*	0.31 (−0.32, 0.95), $P = 0.33$
Tr				
IL-10	1.70	1.63	0.12 (−0.35, 0.59), $P = 0.6$	0.20 (−0.35, 0.75), $P = 0.5$
Th1				
IFN-γ	1.85	1.39	0.51 (0.06, 0.95), $P = 0.03$*	0.73 (0.17, 1.29), $P = 0.01$*
IL-2	1.59	1.11	0.53 (0.14, 0.92), $P = 0.008$*	0.80 (0.34, 1.25), $P = 0.001$*

*Statistically significant.

Table II. Relationship Between Gulf illness Status as Defined from Stage II and Levels of Polyclonally Activated Cytokine Producing CD4+ Cells

T cells	Symptomatic GWVs (%)	Well GWVs (%)	Mean difference (95% CI) corrected for: Age and gender	Mean difference (95% CI) corrected for: Age, gender, vaccination status, antidepressant use, BDI score, and history of atopic illness
Th2				
IL-4	4.33	3.93	0.44 (−0.41, 1.29), $P = 0.3$	0.41 (−0.57, 1.40), $P = 0.4$
Tr				
IL-10	5.08	3.20	1.84 (1.07, 2.60), $P < 0.001$*	1.93 (0.99, 2.86), $P < 0.001$*
Th1				
IFN-γ	12.0	11.6	0.55 (−1.36, 2.46), $P = 0.6$	−1.63 (−3.79, 0.53), $P = 0.14$
IL-2	17.4	16.4	−1.21 (−3.75, 1.33), $P = 0.3$	−1.83 (−4.72, 1.06), $P = 0.2$

*Statistically significant.

may also be a result of chance in the sampling for this study or could be due to the fact that the current sample is much smaller, and the original effect size modest, and we therefore lack statistical power to detect underlying differences. No single agent, including anthrax and plague vaccines given for protection against possible biological warfare, was associated with significant Th1/Th2 bias.

In this, as in our previous study (12) Gulf war veterans received variable numbers of vaccines and were divided into quintiles on this basis and associations sought between vaccine exposures and levels of stimulated and resting cytokine secreting cells (a total of eight analyses). There was a weak but significant association between reducing levels of polyclonally activated IFN-γ+ Th1 cells and increasing total vaccine exposure ($P = 0.03$) (Fig. 3). However, although there was a trend for increasing levels of polyclonally activated IL-4+ cells with vaccine exposure, this failed to reach statistical significance and all

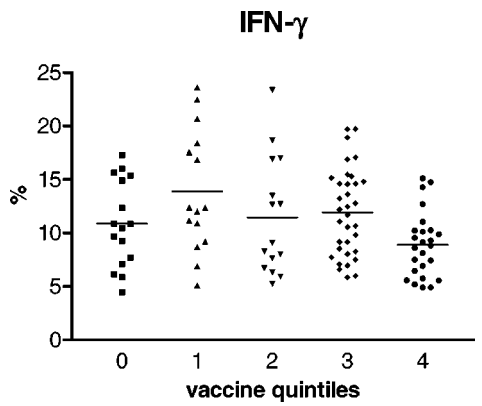

Fig. 3. Effects of number of vaccines on Th1/Th2 cytokines in Gulf War veterans. Graph shows percentage of polyclonally activated Th1 (IFN-γ+) cells in Gulf War veterans divided into quintiles according to the number of vaccines received. A significant trend for decreasing Th1 cells and increasing numbers of vaccines is seen ($P < 0.05$).

analyses for the other cytokines were similarly negative. It is possible, therefore, that the association detected between vaccine exposure and IFN-γ secretion is the result of a Type 1 error.

DISCUSSION

The present study was designed to examine whether alterations in cellular immune activation are present in symptomatic veterans of the first Gulf War, whether any changes are symptom specific, and whether exposures such as multiple vaccines could account for the observations. Specifically, we also aimed to test the existing Rook and Zumla hypothesis (13) that Gulf war-related multisymptom illness resulted from an immune balance biased toward Th2, as a result of multiple vaccines, the use of pertussis as adjuvant, or biological warfare vaccines given under conditions of operational stress.

Despite the fact that our analyses were performed 9 years after the original conflict, and several years after symptoms arose, we were able to detect significant differences in immune activation in symptomatic Gulf War veterans, compared with their well counterparts. Through the analysis of cytokine production by nonstimulated cells, we were able to demonstrate that veterans with multisymptom ill health were distinguished from their asymptomatic counterparts in having a low-grade, ongoing immune activation with the characteristics of a Th1 type. In addition, we demonstrate that CD4+ lymphocytes secreting IL-10 after stimulation *in vitro* are specifically expanded in the symptomatic Gulf War veteran group. *In vitro* polyclonal stimulation is designed to reveal the full potential of cytokine production by effector memory lymphocytes in a given individual, and thus our finding suggests that veterans with multiple symptoms have at some stage been exposed to an internal or external environment in which the generation of IL-10-producing CD4+ cells is

promoted. Higher levels of nonstimulated IL-4 producing cells were also seen in sick Gulf War veterans. In the comparison with well Gulf War veterans, this difference did not survive correction for clinical features (vaccination status, antidepressant use, mood, and history of atopic illness) in our multiple regression analysis. In the comparison with sick Bosnia/Era veterans, nonstimulated IL-4 producing cells were significantly elevated, suggesting that Gulf deployment itself may have had effects on the quality of the ongoing immune activation.

There have been numerous previous studies on immune function in Gulf War veterans, largely failing to show an effect of deployment on immune function (reviewed in Ref. 26). However, our study has several key advantages. First, to our knowledge, no equivalent studies have examined an equally large number of cases; no studies have been nested within large epidemiological analyses capable of examining the relationship to illness without self-referral bias; and none have used the approach of detecting cytokine producing cells directly by flow cytometry. This technique has the advantage that single cytokine secreting CD4 cells are visualized and counted directly. In contrast, studies measuring levels of cytokines in serum or in supernatants, or levels of cytokine mRNA, following polyclonal stimulation are unable to identify the cellular source of the protein. Since cultured macrophages and NK cells also produce the cytokines IFN-γ, IL-4, and IL-10 (24), it is probable that such approaches lack the sensitivity required to detect subtle changes in numbers of cytokine producing cells and would not be able to examine the specific hypothesis that Th balance is abnormal. Other investigators have used cryopreserved lymphocytes, which generally have an inferior performance compared to fresh cells in functional assays (27).

One of our specific aims was to examine formally the hypothesis that Gulf War-related illness is associated with a Th2 bias in cytokine balance (13). This proposal states that the Th2 bias results from exposure to multiple vaccines, pertussis, or stress. In a previous epidemiological study on the same cohort, we found a link between multisymptom illness and multiple vaccine exposure and demonstrated that illness was a more likely outcome if vaccines were given in the war zone, supporting an additional role for stress (12). In the present study, we could not find robust evidence that multisymptom illness is associated with Th2 bias; if anything, the bias we observe in sick veterans is Th1 or Th0. Furthermore, in a previous study we were unable to find an association between multisymptom illness in veterans and the presence of antinuclear autoantibodies as a surrogate marker for Th2 bias (28). The weak but significant relationship between increasing vaccine numbers and declining IFN-$\gamma+$ Th1 cells should be interpreted with caution, since a clear reciprocal trend for increasing numbers of IL-4 secreting Th2 cells with increasing vaccines was not observed. Data on vaccination were also incomplete and supplemented with self-reported exposure, which may be prone to bias (29), rather than antivaccine antibody measurement. The specific hypothesis that multiple vaccination favors a Th2 cytokine bias may be more amenable to testing in prospective studies. In terms of other possible Th2 biasing effects, stress exposure is difficult to quantify, but we were able to exclude pertussis or any other single vaccine agent, including anthrax, as being associated with Th2 bias.

The association between multisymptom illness, seen in Gulf War veterans and nondeployed/Bosnia veterans, and elevated levels of polyclonally stimulated IL-10 producing cells is particularly striking. IL-10 was originally described as a cytokine produced by Th2 cells, although it is now apparent that other cells can produce IL-10, including regulatory T cells (30). We were unable to demonstrate a clear correlation between IL-4 or IFN-$\gamma+$ and IL-10 producing cells, suggesting that these constitute three distinct populations of effector cells in symptomatic veterans. IL-10 mediates potent antiinflammatory effects, earning it the term "suppressor of systemic pathology." In animal models IL-10 protects against severe inflammatory pathology associated with infection and contributes to impaired clearance of infectious agents such as *Leishmania* (30). Elevated IL-10 levels are known to result from antidepressant medication (25) but use of antidepressant and antianxiety medication was not significantly different between our study groups, and controlling for their usage did not alter our findings. Irrespective of the mechanism of induction of high levels of IL-10 producing CD4 cells, our finding raises considerations in relation to vaccination during deployment. For example, high IL-10 levels during the deployment period, when vaccination is being carried out, could have unwanted effects on the induction of protective immunity, an issue that could be addressed in prospective studies.

Our demonstration that veterans have a Th1-like, low-level, chronic immune activation raises the question whether this finding could be of clinical relevance. It is unlikely that such a nonspecific marker could be used as a diagnostic measure, particularly since there was considerable overlap in levels of IFN-γ across the study groups. However, levels of Th1-like cells could be used in the future to monitor the effects of intervention therapies, which are being increasingly applied in veterans with multisymptom illness (31). The evidence of a chronic Th1 bias also raises the question whether this will have any long-term health consequences, since diseases such as organ-specific autoimmunity have been associated with Th1 bias

(32). Again, this possibility will require evaluation during prospective follow-up, but to date there is no evidence to support this possibility.

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