Reprinted from the International Journal of Medicine 1998; 1: 80-92.

Mycoplasmal Infections and Fibromyalgia/Chronic Fatigue Illness (Gulf War Illness) Associated with Deployment to Operation Desert Storm

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Abstract

Servicemen and servicewomen who served in the Persian Gulf region during Operation Desert Storm returned to the U.S. and slowly presented with chronic illnesses that produce complex signs and symptoms, such as polyarthralgia, chronic fatigue, short-term memory loss, sleep difficulties, headaches, intermittent fevers, skin rashes, diarrhea, vision problems, nausea, breathing and heart problems and other signs and symptoms that are collectively called Gulf War Syndrome or Gulf War Illness. Although there is not yet a case definition for Gulf War Illness, the chronic signs and symptoms loosely fit the clinical criteria for Chronic Fatigue Syndrome (CFS) and Fibromyalgia. Using the technique of Nucleoprotein Gene Tracking, a cDNA hybridization technique that utilizes isolated nucleoproteins for probing with unique cDNA sequences, slightly under one-half (45%) of these soldiers and their immediate family members (76/170) who have Gulf War Illness signs and symptoms showed evidence of mycoplasmal infections in their blood leukocytes, but not in their blood plasma or serum. In contrast, in nondeployed, healthy adults the incidence of mycoplasma-positive tests were <5% (2/41). Mycoplasma-positive cases of Gulf War Illness-CFS have been successfully treated with multiple cycles of doxycycline (200-300 mg/d), ciprofloxacin (1500 mg/d) or azithromycin (500 mg/d). All patients on antibiotic therapy (n=73) relapsed within weeks after the first cycle of therapy, but 7/73 recovered after 2 cycles of therapy, another 12/73 after 3 cycles, another 18/73 after 4 cycles, another 21/73 after 6 cycles of therapy and 14/73 are still undergoing therapy. Gulf War Illness patients who recovered from their illness after several (3-7) 6-week cycles of antibiotic therapy were retested for evidence of mycoplasmal infection and were found to have reverted to a mycoplasma-negative phenotype. We conclude that a subset of Gulf War Illness patients have mycoplasmal and possibly other chronic bacterial infections, and treatment with appropriate antibiotics can result in recovery from this chronic condition.

Introduction

Gulf War Syndrome or Gulf War Illness (GWI) is a term that has been used to describe a collection of chronic signs and symptoms reported by U.S., British and other coalition forces in soldiers that were deployed in Operation Desert Storm (ODS). Approximately 80,000-100,000 veterans of Operation Desert Storm returned in 1991 from the Persian Gulf and slowly (6-24 months) presented with a variety of GWI signs and symptoms characterized by disabling fatigue, intermittent fevers, night sweats, arthralgia, myalgia, impairments in short-term memory, headaches, skin rashes, intermittent diarrhea, abdominal bloating, chronic bronchitis, photophobia, confusion, transient visual scotomata, irritability and depression and other signs and symptoms [1, 2]. Although it has been generally accepted that many Gulf War veterans do have medical problems, the signs and symptoms of GWI are not well established as criteria for particular illnesses, and they do not readily fit into the common diagnosis categories used by the Department of Defense or Department of Veterans' Affairs medical facilities [3, 4]. This has resulted in unknown diagnoses, or GWI patients have been diagnosed with psychological problems, such as Post Traumatic Stress Disorder (PTSD) [1]. Most physicians and scientists that work on GWI do not accept that this disorder can be explained by psychiatric diagnoses or successfully treated as somatization disorders [5].

That veterans with GWI have chronic illnesses at higher rates than military personnel from the same units that were not deployed to the Persian Gulf Theater of Operations was carefully shown in a case control study performed by the Center for Disease Control [6]. In these units hundreds of soldiers or airmen that were deployed to the Persian Gulf region were compared to similar numbers of soldiers or airmen from the same units that were not deployed (Figure 1). In the four units that they studied, the Gulf-deployed soldiers had a variety of chronic illness symptoms that were not present in the same frequencies in the undeployed soldiers [6]. Some of the ODS veterans that have the multiple chronic symptoms shown in Figure 1 may eventually have their diagnoses linked to chemical exposures in the Persian Gulf, such as oil spills and fires, smoke from military operations, chemicals on clothing, pesticides, chemoprophylactic agents, chemical weapons and others [1, 5]. In some cases, such exposure may have resulted in Multiple Chemical Sensitivity Syndrome (MCS). MCS shares some but not all of the symptoms associated with GWI [1, 2, 5, 6]. Moreover, the spread of the illness to immediate family members is not consistent with a diagnosis of MCS [5].

Medical explanations for GWI have remained elusive. Parasites such as *Leishmaniasis* and bacteria such as *Cholera* are endemic to the Middle East and could be the cause for illness in at least some of the soldiers with GWI. Diagnostic tests are available for many of these agents, however, and there have been no reports that they are the causes for symptoms in a large numbers of patients with GWI. In some cases the explanation has been attributed to known parasitic diseases, such as

infections by *Leishmania tropica*, probably spread by the sandfly *Phlebotomus papatasi*. This can result in viscerotropic Leishmaniasis [7]. However, many of the common signs and symptoms of GWI do not fit with this explanation, and diagnosis of Leishmaniasis is uncommon in Gulf War veterans. Nonetheless, it is unclear how prevalent Leishmaniasis infections are in GWI patients.

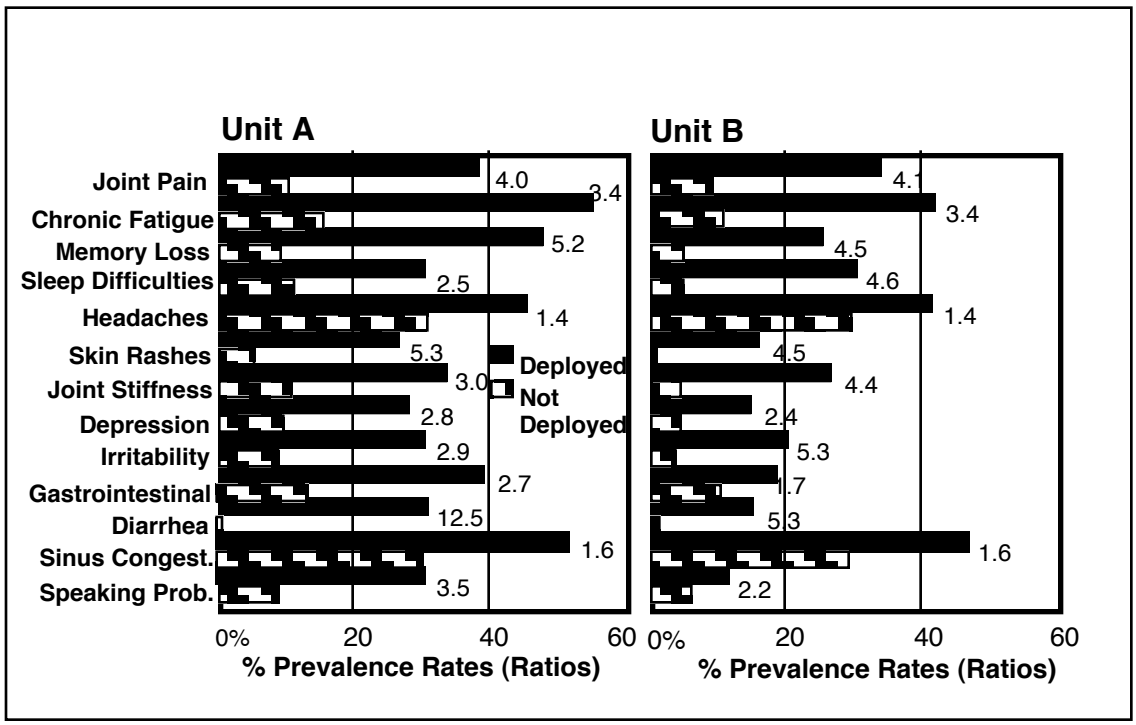

Figure 1. Control study on Gulf War illnesses conducted by the CDC. The bars indicate the frequencies of common symptoms in deployed (solid bars) and non-deployed (stippled bars) members of Air National Guard (Unit A) and Air Force Reserve (Unit B) units where approximately equal numbers of airmen were deployed or not deployed to the Arabian Gulf region. The numbers by the solid bars indicate the ratio of prevalence (data from reference 6).

The variable incubation time of GWI, ranging from months to years after presumed exposure, the cyclic nature of the relapsing fevers and the other chronic signs and symptoms and their appearance in immediate family members are consistent with a disease caused by biological agent(s). We have proposed that many of the signs and symptoms of GWI may be caused by chronic host responses to infectious agents resulting in cytokine production and a variety of other responses that result in a Fibromyalgia (FM)- or Chronic Fatigue Syndrome (CFS)-like disorder [2, 8-10]. When the signs and symptoms of GWI were compared to the literature signs and symptoms of FM/CFS [11, 12], the similarity of these disorders was striking (Table 1) [2, 13]. The classical working case definition of CFS is that of Holmes et al. [14] who proposed that CFS is primarily characterized by persistent or relapsing, debilitating fatigue or easy fatigability in a person who has no previous history of similar symptoms, that does not resolve with rest and is severe enough to reduce or impair average daily activity below 50% of the patient's premorbid activity level. In addition to the absence of clinical conditions that could easily explain the symptoms, such as malignancies or autoimmune diseases, patients present with mild fever, sore throat, arthralgia, myalgia, generalized muscle weakness, headaches, painful lymph nodes, sleep difficulties, and neuropsychologic complaints, such as memory loss, photophobia, confusion, transient visual scotomata, irritability and depression, closely paralleling those found in GWI (Figure 1). This indicates that GWI is not a separate syndrome; it is a CFS- or CFIDS-like disorder [2, 8-10]. The signs and symptoms of GWI also overlap with FM, a condition similar to CSF or CFIDS but one that has polymyalgia as its major sign/symptom.

In support of a biological hypothesis for a subset of patients with GWI, infectious agents have been found in GWI patients' urine [15] and blood [9, 10]. Using a microscopic technique for determining bacterial infections in urine, Hyman [15] has found that many Gulf War veterans have evidence of bacterial infections that can be successfully treated with several courses of broad spectrum antibiotics. We found that most of the GWI/FM/CFS symptoms can be explained by chronic pathogenic mycoplasmal infections [9, 10]. Mycoplasmal infections usually produce relatively benign diseases limited to particular tissue sites or organs, such as urinary tract or respiratory infections. However, the types of mycoplasmas that we have detected in Desert Storm veterans, such as *Mycoplasma fermentans* (incognitus) [10] that may be causing the chronic fatigue and other signs and symptoms, are very pathogenic, colonize a variety of organs and tissues, and are difficult to treat [16]. These mycoplasmas are not easily detected but can be identified by a technique that we developed called Nucleoprotein Gene

Tracking [17]. In our pilot study on 30 veterans with GWI/FM/CFS and their families, we have found evidence of mycoplasmal infections in about one-half of the GWI patients' blood leukocyte samples [10]. In the present study we have extended our preliminary findings that GWI patients and their immediate family members with GWI/FM/CSF show evidence of mycoplasmal infections that can be successfully treated with antibiotics.

Table 1. Commonly found signs and symptoms of Gulf War Illness/Fibromyalgia/Chronic Fatigue Syndrome.

Major Signs and Symptoms (50-100% of patients)

Arthralgia, Chronic fatigue, Polymyalgia, Memory loss, Night sweats, Headaches, Skin rashes, Intermittent fever, Sleep disturbances, Concentration loss, Muscle spasms, Diarrhea, Breathing problems and congestion, Vision problems, Photophobia, Gastrointestinal problems, Heart plapitations, Shortness of breath, Hives, Sinus congestion and/or pain

Minor Signs and Symptoms (25-50% of patients)

Depression, Nervousness, Anxiety, Chest pain, Dizziness, Nausea, Stomach pain, Vertigo, Hair loss, Urination problems, Chemical sensitivities, Frequent coughing, Bleeding gums, Sex problems, Eye redness, Eye pain, Eye drying, Cracking skin, Numbness in limbs, Abdominal bloating.

Methods

Preparation of Blood Samples

Blood is obtained from control and GWI patients by certified medical technicians, nurses or physicians. A Blood Disclosure Form must be completed before blood is accepted. Samples are coded, and clinical information from our Gulf War Survey Form is entered into a spreadsheet for future analysis. Blood (15-20 cc) is drawn into citrated tubes, mixed and shipped to the Institute for Molecular Medicine in Irvine, CA by overnight courier. The blood is allowed to settle overnight at 4°C, and some plasma (500 µl) is removed and saved. The blood is remixed and an aliquot (500 µl) is removed before adding 10 ml of phosphate-buffered saline (PBS) and underlayering with Histopaque 1077 (Sigma, St. Louis, MO). After centrifuging for 30 min at 400 x g, the opaque interface containing the mononuclear cell fraction is removed by pipette to a new tube, PBS is added and the cell suspension is washed by centrifugation for 10 min at 750 x g. The pellet is suspended in 5 ml of RSB (0.01 M NaCl, 0.0015 M $MgCl_2$, 0.01 M Tris-HCl, pH 7.4) by vortexing, and an aliquot (~2 x 10^5 leukocytes) is removed prior to incubation for 10 min at room temperature to remove remaining erythrocytes by osmotic lysis. The mixture is then centrifuged for 10 min at 750 x g, and the cell pellet is vortexed in 5 ml of RSB containing 0.04% NP-40, and is centrifuged again for 10 min at 750 x g. The supernatant is then discarded after saving a 500 µl aliquot. The nuclear pellet is resuspended in 1 ml of K buffer (0.06 M KCl, 0.015 M NaCl, 0.01 M $MgCl_2$, 1 mM $CaCl_2$, 0.015 M Tris-HCl, pH 7.5) containing 20% glycerol. After the NP-40 buffer wash, two more washes in the same buffer without NP-40 are performed, as before. Using this procedure mycoplasma inside cells will fractionate with nuclei, or they are present in nuclei or at perinuclear sites (as has been seen by J. Baseman, personal communication, using confocal microscopy). Fifty µl of the nuclear fraction is saved, and all samples are stored at -70°C.

Gene Tracking Methods

The preparation of the six nucleoprotein complex (NPC) fractions from leukocyte nuclei of GWI patients has been described previously [10, 17] (Figure 2). Prior to restriction digestion with *Msp*I, nuclei are washed in the K buffer. The initial *Msp*I digestion of nuclei is performed for 1 h using 1600 units enzyme/mg nuclear protein. At the completion of this digestion, the first precursor NP complex fraction, S1, corresponding to the supernatant, is generated by microcentrifugation for 1 min at 12000 x g. The remaining pellet is then washed in TM buffer (0.01 M $MgCl_2$, 0.10 M Tris-HCl, pH 7.4) by resuspension followed by vortexing and subsequent microcentrifugation for 1 min at 12,000 x g. The resultant supernatant from this wash step (M1) corresponds to the second precursor NPC fraction generated by this method. At this phase in the precursor NPC fractionation, the pellet following the microcentrifugation and resuspension in K buffer is redigested with *Msp*I at 50% of the initial enzyme concentration for 30 min. The second digestion is followed by microcentrifugation at 12,000 x g to generate the third precursor NP complex fraction supernatant (S2). Microcentrifugation and resuspension in TM buffer is then repeated to generate the fourth precursor NPC fraction (M2). The resultant pellet is subjected to an additional microcentrifugation and resuspension in K buffer diluted 10-times to yield the fifth precursor NPC fraction (0.1 K). The final pellet is then resuspended in K buffer which had been diluted 10-times by aspiration and vortexing to distribute the residual material uniformly to yield NPC fraction R. At each step in the direct *Msp*I digestion of nuclei, aliquots of the preparation are monitored by microscopy for morphological integrity of the remaining nuclei.

Native low ionic strength electrophoresis of the six NPC fractions isolated from the blood leukocytes of a GWI patient was conducted and the separated NPC fractions were transferred to Nytran for probing using a mycoplasma-specific probe [10].

Native low ionic strength electrophoresis is performed using a modification of electrophoretic systems designed to fractionate ribonucleoproteins and deoxyribonucleo-proteins [17]. Samples from precursor NPC fractions to be separated by native low ionic strength electrophoresis are diluted with TB buffer (0.010 M. Tris-HCl, 0.010 M boric acid, pH 7.8). Chelating agents were excluded from all buffers. Low-ionic strength electrophoresis is performed on a minigel apparatus (Horizon^{TM}, Model 200, Bethesda Research, Bethesda, MD) using 1% ultrapure agarose (Bethesda Research) in TB buffer at 75 mV for approximately 1 h. The precursor NPC fractions are visualized by ethidium bromide staining (1 mg/ml) under ultraviolet irradiation prior to transfer. After transfer to Nytran, the partially purified NPC can be probed with a mycoplasma-specific probe. Once the blot has been exposed to X-ray film, it can be stripped and reprobed with another specific probe [17].

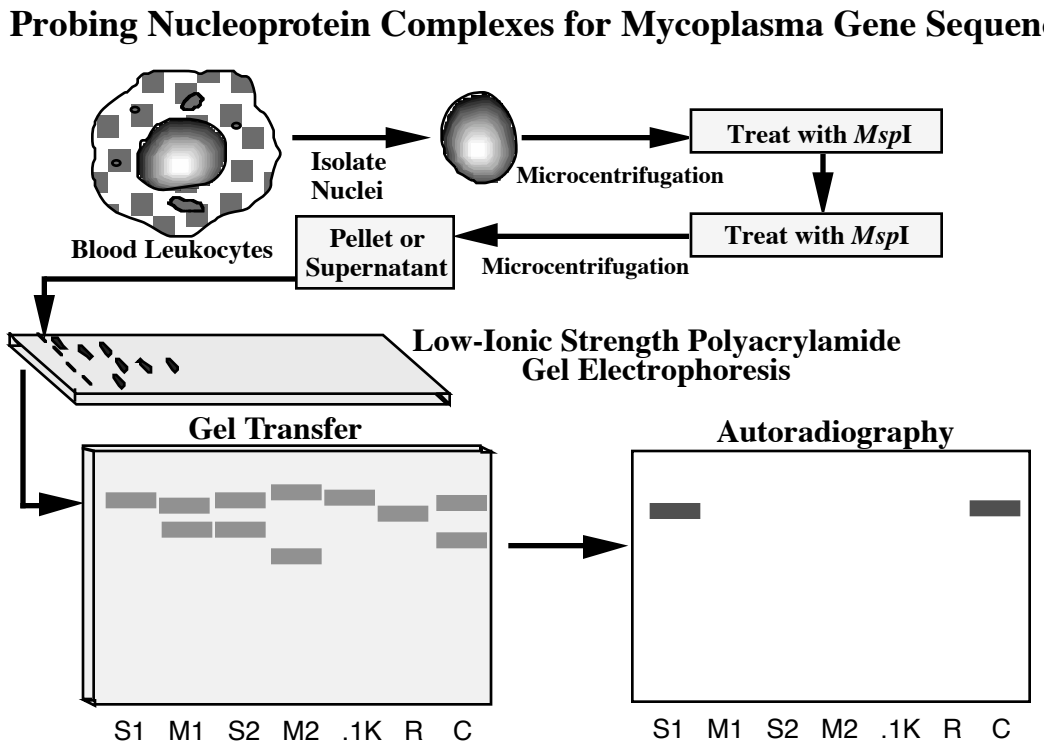

Figure 2. Flow chart depicting steps in the purification of subnuclear nucleoprotein complex fractions fractions and probing for mycoplasma gene sequences. Nuclei were isolated from blood leukocytes and digested according to the procedures described in Methods to generate 6 subnuclear chromatin nucleoprotein complex fractions fractions (S1, M1, S2, M2, 0.1K and R). The nucleoprotein complex fractions fractions were separated by low ionic strength gel electrophoresis with or without DNase-I treatment. The purified nucleoprotein complex fractions fractions were removed from the low ionic strength gel by electroblotting onto Nytran paper, hybridized with ^{32}P-labeled mycoplasma gene probes and subjected to autoradiography.

Results

In patients with GWI/FM/CFS, Nucleoprotein Gene Tracking starts with the preparation of the nucleoprotein complex fractions from leukocyte nuclei of patients' blood (Figure 2). Once the blood leukocytes are obtained, their nuclei are prepared and exposed to a restriction enzyme, *Msp*I, that cuts DNA at specific sites to release specific nucleoprotein complexes. The nucleoprotein complexes are then separated by native (non-denaturing) low-ionic strength gel electrophoresis in the absence of chelating agents and transferred onto a Nytran paper support. Once they are immobilized on the Nytran paper, they can be probed using a radioisotope-labeled (usually ^{32}P-labeled) oligonucleotide that will only bind to a specific DNA sequence. In the example shown in Figure 3, we have probed the nucleoprotein fractions obtained from the blood leukocytes of Subject A with an oligonucleotide probe specific for *Mycoplasma fermentans*. In Figure 3 a mycoplasma-specific gene was found in nucleoprotein fraction S1 purified from the leukocyte nuclei of a GWI patient. This suggests that the mycoplasma has penetrated deep into the nucleus of the patient's blood cells. This subject was placed on several 6 week cycles of doxycycline (200 mg/d) and eventually recovered and his signs and symptoms abated. When retested for mycoplasmal infection in his leukocyte blood fraction, he reverted to a mycoplasma-negative phenotype (data not shown).

In our preliminary study on U.S. veterans with GWI/FM/CFS and their immediate family members with GWI, we have found evidence of mycoplasmal infections in a little under one-half (76/170 or ~45%) of the GWI patients' blood leukocytes.

We also detected mycoplasmal infections in 2 out of 2 British veterans with GWI-CFS. In contrast, in nondeployed, healthy adults the incidence of mycoplasma-positive patients was <5% (2/41). All of the GWI/FM/CFS patients in our group were very ill, so it is unlikely that slightly less than one-half of all Desert Storm veterans with GWI-CFS signs and symptoms have mycoplasmal infections. Thus the final incidence of mycoplasmal infections will likely be lower than ~45% of all GWI patients. In addition, not every Desert Storm veteran had the same type of mycoplasma gene sequences inside their leukocytes. So far, the majority (about 2/3) of the mycoplasma gene sequences that have been identified in GWI patient's blood are characteristic of *Mycoplasma fermentans*. As discussed above, pathogenic invasive mycoplasmas, such as *M. fermentans* or *M. penetrans,* should be treatable with multiple courses of antibiotics, such as doxycycline [8-10, 16]. The majority of those Desert Storm veterans who presented with most of the GWI-CFS symptoms listed in Table 1 had good responses with doxycycline (200-300 mg/day for 6 weeks per course), and after multiple courses of antibiotics (3-7 courses) eventually recovered. Using the technique of Nucleoprotein Gene Tracking, a cDNA hybridization technique that utilizes isolated nucleoproteins for probing with unique cDNA sequences, slightly under one-half (45%) of these soldiers and their immediate family members (76/170) who have Gulf War Illness signs and symptoms showed evidence of mycoplasmal infections in their blood leukocytes, but not in their blood plasma or serum. In contrast, in nondeployed, healthy adults the incidence of mycoplasma-positive tests were <5% (2/41).

Mycoplasma-positive cases of GWI/FM/CFS have been successfully treated with multiple cycles of doxycycline (200-300 mg/d for 6 weeks per cycle), ciprofloxacin (Cipro, 1500 mg/d for 6 weeks per cycle) or azithromycin (Zithromax, 500 mg/d for 6 weeks per cycle). Since the mycoplasmas found in Gulf War Illness patients are intracellular, slowly growing chronic microorganisms, brief treatments with antibiotics (or use of lower doses) are not considered as effective. All of the GWI patients on antibiotic therapy (73/76) relapsed within weeks after the first cycle of therapy, but 7/73 recovered after 2 cycles of therapy, another 12/73 after 3 cycles, another 18/73 after 4 cycles, another 21/73 after 6 cycles of therapy and 14/73 are still undergoing therapy. Some of the recovered GWI/FM/CFS patients (n=19) were retested for the presence of mycoplasma gene sequences in their blood leukocytes. After recovery, we could no longer detect mycoplasma in their blood leukocytes. During recovery we also suggested that these patients practice pollutant avoidance and begin vitamin and nutrient supplementation and physical therapy.

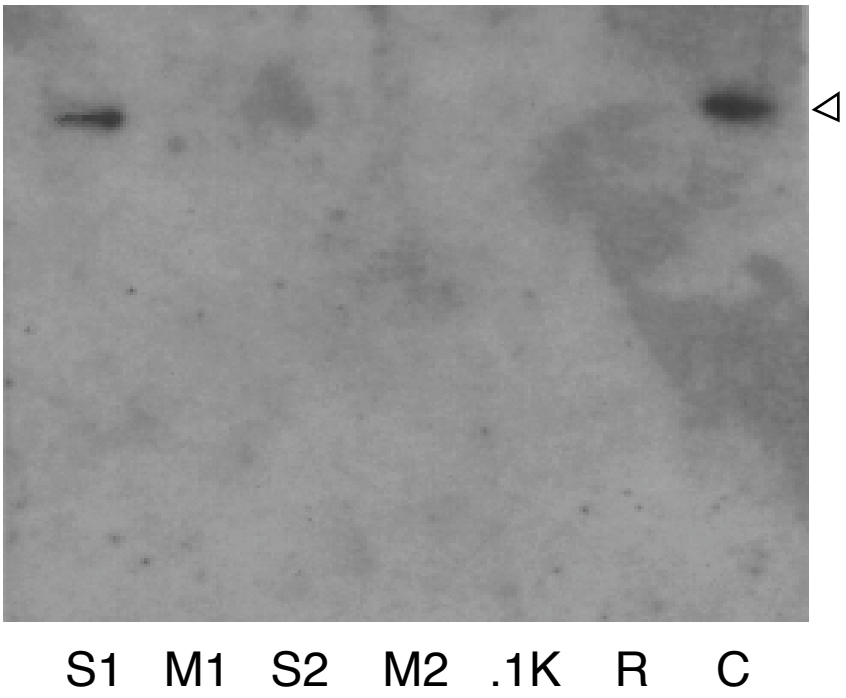

Figure 3. Identification of mycoplasma gene sequences by Gene Tracking in a blood leukocyte cell sample taken from a subject (Subject A) who served in Operation Desert Storm, and who had GWI-CFS. Depected is an autoradiogram that shows the binding of a radioactive probe against a specific mycoplasma DNA sequence in *Mycoplasma fermentans* to isolated leukocyte fraction nucleoproteins. The arrow shows the expected specific binding position of radioactive probe against the mycoplasma genetic sequences in nucleoprotein fraction M1. The positive control is shown in lane C. The results indicate that this soldier is infected with *Mycoplasma fermentans*.

Case Descriptions

Subject A. Subject A was a U.S. Air Force officer attached to the 5th Special Forces Group based at King Fahd Airport west of Dhahran and the 160th Special Operations Unit at King Khalid Military City. He was involved in the Special Forces operations in Kuwait and Iraq. After his return to the U.S., he noticed that he had a constant sore throat, night sweats, fever, shortness of breath, dizziness, joint pain, short term memory loss, vision problems, diarrhea and other bowel problems, skin rashes and severe to moderate fatigue. He eventually left the Air Force and could not obtain treatment from Veterans hospitals for his GWI/FM/CFS. He tested positive for *M. fermentans,* received four courses of doxycycline and has completely recovered. Upon his recovery, he was retested for *M. fermentans* infection by Nucleoprotein Gene Tracking, and he had reverted to mycoplasma-negative.

Subjects B, C and D. Subject B was a U.S. Army officer who served with the 101st Airborne Division (Air Assault). He was deployed on the deep insertions into Iraq. His unit did not come under enemy fire, and he considered his service relatively uneventful, until months after he returned to the U.S. What started out as a relative benign series of flu-like illnesses became progressively worse with intermittent fever, coughing, nausea, gastrointestinal problems, skin rashes, joint pain, memory loss, vision problems and severe headaches. Within 6 months after he presented with GWI/FM/CFS, his wife began to have chronic fatigue and gynecological problems, aching joints, headaches, and her stomach began to swell, causing severe pain. Their 7 year-old daughter then became ill with similar flu-like symptoms that did not go away and progressively became worse, resulting in chronic fatigue, skin lesions, vomiting, headaches, aching joints, and inability to gain weight. She was diagnosed with 'failure to thrive.' Several other families of Gulf War veterans at his base had similar health problems. These families were being told that their symptoms were the result of psychological problems (PTSD), but their symptoms were more consistent with GWI/FM/CFS. Subject A and his entire family tested positive for *M. fermentans* infection and were placed on several 6 week cycles of doxycycline. The entire family has recovered.

Subject E. Subject E is a U.S. Navy Special Forces (SEAL) officer now in Delta One at Fort Bragg, North Carolina, U.S.A. He was in charge of the SEAL units that were involved in covert missions during ODS. He presented after the Gulf War with chronic fatigue, fever, stomach cramps, joint pain, skin rashes, memory loss, dehydration, headaches, heart pain and other symptoms. His vision became so diminished that physicians at Womack Army Hospital in North Carolina were considering surgery. He was never tested for mycoplasmal infections, but after several 6 week courses of doxycycline, he completely recovered.

Subject F. Subject F is a U.S. Air Force nurse who served in the medical evacuations units that operated at several locations in the Persian Gulf, but was not near combat areas. She presented with chronic fatigue, intermittent fever, stomach cramps, joint pain, skin rashes, memory loss, headaches, severe minstrel problems, uterine swelling and other symptoms. She tested positive for mycoplasmal infection and was placed on ciprofloxacin and then doxycycline for several 6 week cycles. She has almost completely recovered.

Subject G. Subject G was a U.S. Army noncommissioned officer assigned in a support unit in Saudi. He presented with chronic fatigue, joint pain, skin rashes, memory loss, severe headaches and heart pain and other signs and symptoms. He was admitted to the Coronary Care Unit of a Major Medical Center with a massive heart attack. Echocardiograms indicted significant loss of heart function, and he was scheduled for a heart transplant. While awaiting a donor in the CCU, he was tested for mycoplasmal infection. He tested positive and was placed on iv doxycycline therapy, followed by oral doxycycline for several months. He responded to the antibiotics, and his echocardiograms showed significant improvement in heart function. He eventually recovered, was released from hospital care and has returned to work.

Discussion

We consider it quite likely that many of the Gulf War veterans suffering from the GWI/FM/CFS signs and symptoms may have been infected with microorganisms, quite possibly pathogenic invasive mycoplasmas and other pathogens (possibly other types of bacteria that cause chronic signs and symptoms, such as *Brucella* sp.). This would also explain the apparent moderately contagious nature of GWI in some veterans, as evidenced by the appearance of similar GWI/FM/CFS symptoms in their immediate family members and their successful treatment with antibiotics that are effective (but not those that are not effective) against mycoplasmal infections. We have suggested that these infections can be treated with doxycycline (200-300 mg/day for each 6 week cycle), ciprofloxacin (Cipro, 1500 mg/day for each 6 week cycle) or azithromycin (Zithromax, 500 mg/day for each 6 week cycle) [8-10]. In some cases it was necessary for physicians to change antibiotics when resistance became a problem. For pediatric use zithromycin is recommended; however, in adolescents (< 8 years old) the use of doxycycline at reduced dose levels has also proven effective. Interestingly, in patients that received antibiotics that are not effective against mycoplasmal infections, such as the penicillins, the GWI/FM/CSF symptoms worsened. In some cases, administration of penicillin-based antibiotics resulted in severe adverse reactions or anaphylaxis. This indicates that the antibiotics were not causing a placebo effect.

In our preliminary study on Gulf War veterans and their family members with GWI we have found evidence of mycoplasmal infections in slightly under one-half of the patients' blood that we have examined. Since this group of patients is considered to be quite ill compared to the average GWI patient, it is likely that the final incidence of mycoplasmal infections in GWI

will be lower than the incidence rate reported here. In addition, not every Gulf War veteran had the same type of mycoplasma DNA sequences inside their white blood cells. Of particular importance, however, was our detection of DNA sequences of infectious origin in the same nucleoprotein complex fractions by the same Gene Tracking technique. Once patients recovered and were able to return to active duty or normal activity, we could no longer detect mycoplasma gene sequences in their blood leukocytes [10]. Preliminary evidence suggests that the *Mycoplasma fermentans* found inside white blood cells of GWI patients may have been modified to make it more pathogenic and more difficult to diagnose. Using the Nucleoprotein Gene Tracking assay we have found unusual gene sequences associated with the same mycoplasma nucleoprotein fraction. For example, we have found HIV-1 *envelope* gene sequences but not the other genes of the HIV-1 virus (unpublished data). Although this preliminary result will require conformation by sequencing the mycoplasma genome in the area of the putative inserted gene, the presence of the HIV-1 *env* gene could explain the unusual pathogenic properties of this mycoplasma and its ability to attach to and enter a variety of cells and tissues and be found in the cells' nuclear fraction. Since we have not detected the other genes of the HIV-1 virus, these mycoplasma-positive GWI patients are not infected with the intact HIV-1 virus. Indeed, although GWI patients possess some of the signs and symptoms of an immunodifficiency syndrome, they do not progress to AIDS, nor do they test positive for intact HIV-1 virus in their serum or plasma (unpublished data). Some GWI patients, however, do test positive (false positive) in some AIDS tests (ELISA) that probe only the gp120 product of the HIV-1 *env* gene. In these patients additional testing for other HIV-1 gene products or enzymes has proved negative, suggesting support for our hypothesis that only the HIV-1 *env* gene and its encoded product are associated with *M. fermentans* infection of the type found in GWI.

Although the FM/CFS-like symptoms in many patients could be the result of chemical exposures as well as chronic infections, this would also be consistent with our findings of pathogenic microorganisms in the blood of slightly under one-half of the soldiers who have GWI/FM/CFS. The remaining patients could have other chronic infections, or their condition may be linked to environmental causes, such as multiple chemical agents that were prevalent in the battlefield. In support of this notion, Abou-Donia and Wilmarth [18] have found that combinations of the antinerve agent pyridostigmine bromide, insect repellent N,N-dimethyl-m-toluamide and the insecticide permethrin produce neurotoxicity, diarrhea, salivation, shortness of breath, locomotor dysfunctions, tremors, and other impairments in healthy adult hens. Haley et al. [19] have analyzed veterans of a U.S. Navy Mobile Construction Reserve Battalion and have found evidence of neurologic injury involving the central, peripheral and autonomic nervous systems in patients with GWI. Although the signs and symptoms of GWI/FM/CFS are complex, Murray-Leisure et al. [20] have described GWI as an illness with major criteria that include pustular dermatitis or skin folliculitis, irritable bowel syndrome or chronic, intermittent diarrhea with abdominal bloating and excess intestinal gas, with or without colonic inflammation, with or without intestinal bleeding and large bone and joint pain plus night sweats. Minor criteria include: heartburn, rectal fissures, bleeding or hemorrhoids, lactose or meat intolerance, splenomegaly and splenic tenderness, weakness and/or chronic fatigue, headaches, muscle aches, polymyalgias, memory loss, hair loss, fevers of unknown origin, unexplained leukocytosis or neutropenia, nasal ulcers or sores, chronic sinus or nasal congestion, atypical chest pain, new-onset asthma or chronic bronchitis, ear infections or tinnitus and dental infections [20]. These criteria are the most complete set of diagnostic information available for GWI/FM/CFS and may ultimately be accepted as the case definition for GWI.

We have made some preliminary observations that the soldiers that were involved in the deep insertions into Iraq and those that were near Saudi and Kuwaiti SCUD B (SS-1) impact sites, particularly those missiles that caused air bursts but not high explosive ground bursts, may be at highest risk for contracting the infections that we feel are a major culprit in GWI/FM/CFS. Our results and those of other investigators on other possible causes of GWI strongly suggest that there are multiple causes for these illnesses, including chemical and biological agents that can cause persistent chronic symptoms of a complex and cyclic nature.

The infection of soldiers with invasive, pathogenic mycoplasmas and other pathogens (bacteria) can produce complex and intermittent signs and symptoms long after exposure. This would also explain the mildly contagious nature of GWI/FM/CFS in some veterans, and the appearance of similar signs and symptoms in immediate family members. In our first study, of the 73 Desert Storm veterans who had the GWI/FM/CFS symptoms listed in Table 1, 55 had good responses with doxycycline, and after multiple courses of antibiotic eventually recovered [8]. Our results and those of other investigators who are examining other possible infectious and environmental agents and their role in GWI/FM/CFS strongly suggest that there are multiple causes for these chronic illnesses, but a sizable fraction of Gulf War veterans (slightly under one-half) may have identifiable chronic infections caused by mycoplasmal and other chronic bacterial infections that can be successfully treated with the appropriate antibiotics [8-10]. With the alarming increase in chronic illnesses reported by the press in the civilian populations of the Persian Gulf area and the Middle East in general, it is quite possible that many of these cases may be caused by chronic infections of the type described in this contribution.

Acknowledgment
These studies were supported by a grant from the Rhodon Foundation for Biomedical Research, Inc., funds from the David M. Bruton Jr. Charitable Trust and private donations from the families of GWI patients.

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