International Journal of Occupational Medicine, Immunology and Toxicology 1996; 5: 83-86.

DIAGNOSIS AND TREATMENT OF MYCOPLASMAL INFECTIONS IN PERSIAN GULF WAR ILLNESS-CFIDS PATIENTS

GARTH L. NICOLSON

Department of Tumor Biology,
The University of Texas M. D. Anderson Cancer Center,
Houston, Texas 77030

NANCY L. NICOLSON

Rhodon Foundation for Biomedical Research,
Kingwood, Texas 77339

1. Address all correspondence to: Prof. Garth L. Nicolson, Department of Tumor Biology, Box 108, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, Texas 77030 USA Phone (713) 792-7481 Fax (713) 794-0209

2. Abbreviations: CFIDS, chronic fatigue/immune dysfunction syndrome; GWI, Gulf War Illness; MCS, multiple chemical sensitivity; NPC, nucleoprotein complex; ODS, Operation Desert Storm; PTSD, Post Traumatic Stress Disorder.

3. Key Words: Chronic fatigue syndrome, Desert Storm, environmental agents, infections

ABSTRACT

Veterans of Operation Desert Storm returned from the Persian Gulf Theater of Operations and developed multiple signs and symptoms characterized by disabling fatigue, arthralgia, intermittent fever, myalgia, impairments in short-term memory, headaches, skin rashes, diarrhea and additional symptoms that have defied a disease case definition but has been called Gulf War Illness (GWI). In a sampling of GWI patients and symptomatic family members (n=30) we have used the technique of Gene Tracking and have found evidence of mycoplasmal infections in the blood leukocytes of approximately one-half of these cases. Mycoplasma-positive patients can be successfully treated with several 6 week courses of doxycycline (200 mg/d) or other antibiotics. Of the 14/30 patients that were mycoplasma-positive, 11/14 completely recovered after multiple cycles of antibiotics and 3/14 are still undergoing antibiotic therapy and relapsing.

INTRODUCTION

Service in Operation Desert Storm/Desert Shield resulted in a variety of chronic health conditions for over 50,000 U.S. participants. These chronic conditions include illnesses that are characterized by multiple signs and symptoms, such as disabling fatigue, intermittent fever, arthralgia, myalgia, impairments in short-term memory, headaches, skin rashes and other symptoms. Although it has been conceded that many Operation Desert Storm (ODS) veterans have medical problems, the overlapping nature of the signs and symptoms of these chronic illnesses (called Gulf War Illness or GWI) are not well established as criteria for particular diseases or common diagnosis categories (NIH Technology Assessment Workshop Panel, 1990). Thus veterans with GWI have been have been diagnosed with psychological problems such as Post Traumatic Stress Disorder (PTSD) or with well recognized illnesses such as asthma or given a diagnosis of unknown disorder.

Part of the confusion of diagnosing GWI may be that collectively the signs and symptoms of GWI do not readily fit into a common case diagnosis (Cotton, 1992; Boaz Milner et al., 1994). Although the concept of a distinct syndrome (GWI or Gulf War Syndrome) peculiar to ODS veterans has been advanced, it has not been proven (Boaz Milner et al., 1994), and the absence of a distinct diagnosis may have delayed effective treatment of GWI (Nicolson et al., 1995).

A variety of causes have been proposed for GWI (Nicolson et al., 1995). Endemic parasites such as *Leishmaniasis* and bacteria such as *Cholera* could result in some of the symptoms of some of the GWI patients; however, tests are available for these infections. There is no published evidence that such endemic parasites or bacteria are causing the signs and symptoms of GWI in a large fraction of patients. Some soldiers may have contracted *Leishmania tropica*, resulting in viscerotropic leishmaniasis (Magill et al., 1993). The most common signs and symptoms of GWI, however, do not fit with this explanation.

GWI is characterized by long and variable incubation times, ranging from months to years after presumed exposure. In addition, many of the symptoms have a cyclic nature, such as the relapsing fevers and other symptoms. Finally, the appearance of GWI signs and symptoms in immediate family members is consistent with a disease caused by biological agent(s) (Nicolson and Nicolson, 1996). Kizer et al. (1995) recently studied several U.S. National Guard and Reserve military units for evidence of health problems associated with ODS service. In this study, the ODS-deployed soldiers had much higher frequencies of chronic health problems than soldiers in the same units who were not deployed during ODS.

The most common signs and symptoms of GWI include aching joints, chronic fatigue, arthralgia, myalgia, memory loss, headaches, sleeping difficulties, skin rashes, loss of concentration, depression, muscle spasms, nervousness, diarrhea, blurred vision, anxiety, problems breathing, chest and heart pain, dizziness, nausea, stomach pain, photophobia, loss of balance, hives, frequent coughing, chemical sensitivities, eye pain and other vision problems and bleeding gums and other dental problems (see Figure 1 of Nicolson et al., 1995). Autoimmune-like symptoms are also seen in some GWI patients. Since immediate family members have also presented with similar signs and symptoms, it is likely that some GWI patients have a transmittable disorder (Nicolson and Nicolson, 1996). Other ODS veterans may eventually have their diagnoses linked to chemical exposures, such as oil spills and fires, smoke from military operations, chemicals on clothing, pesticides, chemoprophylactic agents, chemical weapons and others. In some cases, such exposure may have resulted in Multiple Chemical Sensitivities (MCS). MCS shares some but not all of the signs and symptoms listed above, including immune dysfunction (Vojdani et al., 1992). However, the apparent spread of GWI to immediate family members is not consistent with a diagnosis of MCS. In our study of 650 ODS veterans with GWI, only a portion (~25%) complained of MCS signs and symptoms (Nicolson et al., 1995). Fine sand exposure was rather ubiquitous during the ODS deployment, and continued pulmonary exposure could have resulted in immunosuppression in some soldiers and eventually infection by opportunistic microorganisms. Continuous exposure to fine sand particles (< 1 μm diameter) can result in hyperergic lung conditions, and in more severe cases pneumonitis (Korényi-Both et al. 1996).

Gulf War Illness and Chronic Fatigue/Immune Dysfunction Syndrome

When we compared the 30 most common signs and symptoms of GWI to Chronic Fatigue Syndrome or Chronic Fatigue-Immune Dysfunction Syndrome (CFIDS), there was almost an identical match

(Nicolson and Nicolson, 1996). Others have also noticed that the symptomology of GWI and CFIDS are similar (Schmidt and Blanck, 1995). CFIDS 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 signs and 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, memory loss, photophobia, confusion, transient visual scotomata, irritability and depression (Holmes et al., 1988). Thus the signs of CFIDS and GWI are very similar, indicating that GWI is not a separate syndrome (Nicolson and Nicolson, 1996).

GWI could be caused by host responses to chronic infections, resulting in cytokine production and the signs and symptoms commonly found in GWI and CFIDS patients (Nicolson and Nicolson, 1996). Alternatively, the CFIDS signs and symptoms could be caused by chemical insults (Vojdani et al. 1992). We previously suggested that most of the GWI-CFIDS signs and symptoms could be explained by chronic pathogenic mycoplasma infections (Nicolson and Nicolson, 1995). Most mycoplasma infections, however, produce diseases limited to particular tissue sites or organs, such as urinary or respiratory tracts. Mycoplasmal infections can progress to involve other organs and tissues, and CNS disorders or other pathologies are not uncommon. Moreover, mycoplasmal infections can cause the chronic fatigue and other symptoms of GWI. The responses of GWI patients to antibiotics, such as doxycycline, that are effective against mycoplasmal infections, suggested that such infections could be involved in at least a portion of GWI (Nicolson and Nicolson, 1995).

To determine microorganism infections blood is a good specimen for analysis. Mycoplasmas are not easily detected in blood but can be identified by a technique that we developed called Gene Tracking (Rosenberg-Nicolson and Nicolson, 1992; Nicolson and Nicolson, 1993; 1994; Nicolson et al., 1995). This technique can reveal the mycoplasma nucleic acid by a gene hybridization procedure even if it is tightly complexed with nucleoproteins and present inside cells where detection is difficult by more standard procedures, such as polymerase chain reaction. To demonstrate that microorganisms such as mycoplasmas are present in patients with GWI we initiated an investigation of their blood for the presence of this type of infection. Thus Gene Tracking was employed to confirm the presence of mycoplasma DNA sequences in GWI patients' leukocytes.

METHODS

Blood was obtained from control and GWI patients and Blood Disclosure and Gulf War Illness Survey forms were completed before blood was accepted. Samples were coded, and clinical information from the Gulf War Survey form was entered for future analysis. Blood was drawn into citrated tubes, mixed and shipped to Houston by overnight courier, and allowed to settle overnight at 4°C. The blood (20 cc) was remixed before adding 10 ml of phosphate-buffered saline (PBS) and underlayering with Histopaque 1077 (Sigma, St. Louis, MO). After centrifugation for 30 min at 400 x g, the opaque interface containing the white cell fraction was removed by pipet to a new tube, phosphate-buffered saline (PBS) was added, and the cell suspension was washed by centrifugation for 10 min at 250 x g. The pellet was suspended in 5 ml of RSB buffer (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) removed prior to incubation for 10 min at room temperature to remove remaining erythrocytes by osmotic lysis. The mixture was then centrifuged for 10 min at 1,000 x g, and the cell pellet was vortexed in 5 ml of RSB containing 0.04% NP-40 and centrifuged again for 10 min at 1,000 x g. The nuclear pellet was 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. Six nucleoprotein complex (NPC) fractions from leukocyte nuclei of GWI patients were prepared (Fig. 1). Using this procedure the mycoplasma inside cells will fractionate with nuclei. The nuclei were digested with *Msp*I in K buffer, and the nucleoprotein complex (NPC) fractions S1, M1, S2, M2, 0.1K and R were generated as described elsewhere (Nicolson and Nicolson, 1993; 1994).

After native low ionic strength electrophoresis of the six NPC fractions isolated from the blood leukocytes of GWI patients, the separated NPC fractions were transferred to Nytran for DNA hybridization analysis (Fig. 1). After Nytran transfer, the partially purified NPC were probed with a

mycoplasma-specific probe (see Fig. 2, Gene Tracking blot for mycoplasma-specific gene sequences). The mycoplasma-specific probe was generated from cDNA generously supplied by Dr. Joel Baseman (University of Texas Health Science Center, San Antonio). Mycoplasma-specific probes were generated from *M. fermentans* (incognitus strain), *M. genitalium,* and *M. orale.* Control cDNAs included probes from *A. laidwaii*, a related organism not known as a human pathogen.

RESULTS

Mycoplasmal infections in GWI patients

We used mycoplasma-specific gene probes to determine the presence of mycoplasma gene sequences in the NPC fractions obtained from the leukocytes of GWI patients (n=30) and normal healthy controls (n=21). The Gene Tracking results confirmed that approximately one-half of the GWI patients (14/30) had mycoplasmal infections from the positive mycoplasma gene hybridization signal in the isolated NPC fractions from their blood leukocytes (Fig. 1); whereas none of the normal controls (0/21) contained mycoplasma gene sequences in their leukocyte NPC fractions (data not shown). This assay uses specific mycoplasma DNAs as a positive control. Using hybridization probes for *M. fermentans* (incognitus strain), *M. genitalium, A. laidwaii,* and other mycoplasmas we have been able to confirm that the majority of the mycoplasma-positive patients (~65%) had only one type of infection, *M. fermentans* (incognitus strain).

When mycoplasmal infections were detected, specific treatment suggestions were made to primary care physicians who then evaluated each case and reported their findings. All medical records and test results were kept coded. Since pathogenic invasive mycoplasmas, such as *M. fermentans* (incognitus) or *M. penetrans,* should be treatable with multiple courses of antibiotics, such as doxycycline (200 mg/d) (Lo et al., 1991; Nicolson and Nicolson, 1995) or other antibiotics (ciprofloxacin, azithromycin), specific recommendations were made for each mycoplasma-positive patient. As shown in the next section, a nonscientific sample of ODS veterans with evidence of mycoplasmal infections can be successfully treated. Of the 14/30 patients that were mycoplasma-positive, 11/14 completely recovered after multiple cycles of antibiotics and 3/14 are still undergoing therapy and continuing on antibiotics. Four of the patients that completely recovered after cycles of antibiotic therapy were retested for the presence of mycoplasma gene sequences in their blood leukocytes and were negative for the presence of mycoplasmal gene sequences.

Gulf War Illness Case Descriptions and Treatment Outcomes

Subject A. A 37 year-old U.S. Navy officer in the SEAL Units of the 5th Special Forces was deployed as part of a Joint Special Operations unit into Iraq. Within 9 months after his return to the U.S. he presented with chronic fatigue, skin rashes, diarrhea, headaches, aching joints, muscle pain, fevers, sleep problems, nausea, severe vision problems, vomiting and lost 45 lbs. His illness was worse after flying, diving and physical extreme activity. Using Gene Tracking he tested Positive for *M. fermentans* (incognitus strain) and was placed on doxycycline (6 wk, 200 mg/d) therapy. He completely recovered after four cycles of doxycycline therapy.

Subject B. A 36 year-old U.S. Air Force officer, attached to the 5th Special Forces, was deployed at King Kahlad Military City during Operation Desert Storm. He was exposed to SCUD (SS-1) missile attacks during the ground offensive. Within 6 months after his return to the U.S. he presented with chronic fatigue, skin rashes, diarrhea, headaches, aching joints, muscle pain, fevers, sleep problems, vision problems, nausea, memory loss and stomach pain. Using Gene Tracking he tested Positive for *Mycoplasma genitalium* and was placed on doxycycline (6 wk, 200 mg/d) therapy. He recovered after two cycles of doxycycline, but he still relapses occasionally, especially after flying.

Subjects C and D. A 48 year-old U.S. Marine Corps officer, attached to the Central Command Staff, was deployed in Saudi Arabia at ODS Central Command Headquarters. He examined SCUD (SS-1) missile impact sites. Within 10 months after his return to the U.S. he presented with chronic fatigue, skin rashes, diarrhea, headaches, aching joints, muscle pain, fevers, sleep problems, nausea, vision problems, memory loss and dental problems. Within 28 months after his return his wife became ill with

similar symptoms. Using Gene Tracking both tested Positive for *Mycoplasma fermentans* (incognitus strain) and both were placed on doxycycline (6 wk, 100-200 mg/d) therapy. After two cycles of doxycycline, he completely recovered and his wife is recovering, but still relapses with GWI.

Subject E. A 30 year-old female U.S. Army officer in a Nuclear Biological Chemical (NBC) Unit attached to the 101st Airborne Division was deployed at various locations in Iraq, including suspected NBC and SCUD attacks. Within 6 months after her return to U.S. she presented with chronic fatigue, skin rashes, diarrhea, headaches, aching joints, muscle pain, loss of mobility, fevers, sleep problems, nausea, vision problems, MCS, vomiting and menstrual problems. She required constant sleep, and eventually left the military. Using Gene Tracking she tested Positive for *Mycoplasma fermentans* (incognitus strain) and was placed on doxycycline (6 wk, 200 then 100 mg/d) therapy. After 3 cycles of antibiotic therapy, she fully recovered and has only occasionally a few of the signs and symptoms.

Subjects F, G and H. A 36 year-old U.S. Army officer in the 101st Airborne Division was deployed in Iraq near Base Cobra. Within 16 months after his return to U.S. he presented with chronic fatigue, skin rashes, diarrhea, headaches, hearing problems, aching joints, muscle pain, fevers, sleep problems, vision problems, nausea and short term memory loss. Within 24 months his 32 year-old spouse became sick with similar symptoms, including extreme uterine swelling. Their 7 year-old child also had similar symptoms and failed to gain weight. Using Gene Tracking the entire family tested positive for *Mycoplasma* species, and the adults were placed on doxycycline (6 wk, 200 then 100 mg/d). The child was also placed on doxycycline (6 wk, 50 mg/d) therapy. After six cycles of doxycycline, he completely recovered; his spouse is almost completely recovered. The child completely recovered after 4 cycles of antibiotic therapy and is now gaining weight normally.

Subjects I and J. A 53 year old retired U.S. Army officer present in the Persian Gulf as part of the press corps visited various sites in Saudi Arabia and Kuwait, including SCUD impact sites. Within 24 months after his return to the U.S. he presented with chronic fatigue, skin rashes, memory loss, aching joints, muscle pain, fevers, sleep problems, vision problems, diarrhea, headaches and dental problems. Within 12 months after his return his significant other presented with chronic fatigue, severe joint pain, muscle pain, memory loss, vomiting, headaches, menstrual problems, bloating and sleep problems. Using Gene Tracking both tested positive for *Mycoplasma* species, and they were placed on doxycycline (6 wk, 200 then 100 mg/d). After three cycles of doxycycline, he is completely recovered; she is also almost completely recovered but occasionally relapses.

Subject K. A 29 year-old female U.S. Army soldier in an infantry unit was deployed at various locations in Iraq and Kuwait and was in contact with human corpses. Within 12 months after her return to the U.S. she presented with chronic fatigue, skin rashes, diarrhea, headaches, memory loss, fevers, coughing, heart pain, hair loss, vertigo, dental problems, menstrual problems, aching joints, muscle pain, fevers, sleep problems, nausea severe vision problems and bleeding gums. Within 24 months she was partially paralyzed and required a wheelchair. She was also on oxygen. Using Gene Tracking she tested positive for *M. fermentans* incognitus and was placed on ciprofloxacin (6 wk, 500 mg/d). She attempted to take doxycycline, but her MCS prevented continuing on this antibiotic. After three cycles of ciprofloxacin, she is improving, but not yet fully recovered.

DISCUSSION

Our hypothesis is that a large subset of soldiers with GWI, a CFIDS-like condition, have mycoplasmal infections that can be diagnosed and successfully treated. In our limited sample, we found that approximately one-half of the GWI patients and their immediate family members that have similar GWI-CFIDS signs and symptoms show evidence of mycoplasmal infections. In the GWI-CFIDS patients that tested positive, antibiotic therapy was almost always successful in elleviating most if not all of the CFIDS signs and symptoms, and these patients completely recovered. When some (n=4) of the recovered patients who were free of the CFIDS signs and symptoms were retested for mycoplasma gene sequences in their blood, they were found to be negative. We previously found that in 73 ODS veterans who had most of the usual GWI-CFIDS signs and symptoms found here, 55 had good responses with doxycycline therapy, and after multiple courses of antibiotic eventually recovered (Nicolson and

Nicolson, 1995). Unfortunately, in almost all of these GWI-CFIDS patients the diagnostic tests to demonstrate whether they were mycoplasma-positive or not were not available. With the availability of sensitive mycoplasma tests, it should now be possible to confirm that GWI-CFIDS patients have mycoplasmal infections in their blood and recommend appropriate therapy.

We could only detect mycoplasmal infections in approximately one-half of the GWI-CFIDS patients tested. The remaining patients could have other chronic infections, or their CFIDS signs and symptoms could be due to other causes, such as chemical exposures (Vojdani et al., 1992). Thus there is no simple explanation for GWI-CFIDS; it appears to be the result of multiple causes, as was previously discussed by Nicolson et al. (1995).

The presence of mycoplasmal infections in GWI-CFIDS patients and their responses to doxycycline and other antibiotics that are effective against mycoplasmal infections suggests that at least some of the CFIDS patients that did not serve in the Persian Gulf during ODS may have treatable mycoplasmal infections. We have begun to examine this possibility, and our initial findings are that a subset of CFIDS patients with no relationship to ODS have mycoplasmal infections and respond similar to the mycoplasma-positive GWI-CFIDS patients to antibiotics such as doxycycline (unpublished observations).

We are currently developing additional diagnostic tests (polymerase chain reaction) to detect the presence of different species of invasive mycoplasmas that could be used to aid physicians in the diagnosis and treatment of veterans with GWI-CFIDS and civilians with CFIDS. We hope to extend this to a blinded crossover controlled treatment trial involving two antibiotics and placebo to determine the effectiveness of various antibiotics in the treatment of GWI-CFIDS.

REFERENCES

BELL, D.S. (1994) "Chronic fatigue syndrome update." Postgrad. Med. 96:73-81.

BOAZ MILNER, I., AXELROD, B.N., PASQUANTONIA, J. and SILANPAA, M. (1994) "Is there a Gulf War Syndrome?" JAMA 271:661.

COTTON, P. (1992). "Gulf War symptoms remain puzzling." JAMA 268:2619.

HOLMES GP, KAPLAN JE, GANTZ NM, *et al.* (1988) "Chronic Fatigue Syndrome: A working case definition." Ann. Int. Med. 108:387-389.

KIZER, K.W., JOSEPH, S. and RANKIN, J.T. (1995) "Unexplained illness among Persian Gulf War vetrans in an Air National Guard unit: preliminary report--August 1990-March 1995." Morb. Mort. Week Rep. 44:443-447.

KORÉNYI-BOTH, A.L., KORÉNYI-BOTH, A.L. AND JUNCER, D.J. (1996) "Al Eskan Disease--Persian Gulf Syndrome." Military Med., in press.

LO, S.-C., BUCHHOLZ, C.L., WEAR, D.J., *et al.* (1991) "Histopathology and doxycycline treatment in a previously healthy non-AIDS patient systemically infected by *Mycoplasma fermentans* (incognitus strain)." Mod. Pathol. 6:750-754.

MAGILL, A.J., GROGL, M., FASSER, R.A., WELLINGTON, S., and OSTER, C.N. (1993) "Viscerotropic leishmaniasis caused by *Leishmania tropica* in soldiers returning from Operation Desert Storm." New Engl. J. Med. 328:1383-1387.

NICOLSON, G.L. and NICOLSON, N.L. (1993) "Nucleoprotein complexes from metastatic cells containing oncogenes and tissue-specific genes: A novel method to track genes associated with specific nucleoproteins." Cancer Detect. Prev. 18:31-42.

NICOLSON, G.L. and NICOLSON, N.L. (1995) "Doxycycline treatment and Desert Storm." JAMA 273:618-619.

NICOLSON, G.L. and NICOLSON, N.L. (1996) "Chronic Fatigue Illness and Operation Desert Storm." J. Occup. Environ. Med. 38:14-16.

NICOLSON, G.L., HYMAN, E., KORÉNYI-BOTH, A., LOPEZ, D.A., NICOLSON, N.L., REA, W., URNOVITZ, H. (1995) "Progress on Persian Gulf War Illnesses-Reality and hypotheses." Int. J. Occup. Med. Tox. 4:365-370.

NICOLSON, G.L., TALPAZ, M. and NICOLSON, N.L. (1995) "Interferon-α directly inhibits DNA polymerase activity in isolated chromatin nucleoprotein complexes: correlation with IFN-α treatment outcome in patients with chronic myelogenous leukemia." Gene 159:105-111.

NICOLSON, N.L. and NICOLSON, G.L. (1994) "The isolation, purification and analysis of specific gene-containing nucleoproteins and nucleoprotein complexes." Meth. Mol. Genet. 5:281-298.
NIH Technology Assessment Workshop Panel (1990). "The Persian Gulf Experience and Health." JAMA 272:391-396.
ROSENBERG-NICOLSON, N.L. and NICOLSON, G.L. (1992) Nucleoprotein complexes released from large-cell lymphoma nuclei that contain the *abl* oncogene and RNA and DNA polymerase and RNA primase activities. J. Cell. Biochem. 50:43-52.
SCHMIDT, P. and BLANCK, R.M. (1995) "Gulf War Syndrome and CFS." CFIDS Chron. 8:25-27.
SHAFRAN, S. (1991) "The chronic fatigue syndrome." Amer. J. Med. 90:730-739.
VOJDANI, A., GHONEUM, M., and BRAUTBAR, N. (1992) "Immune alteration associated with exposure to toxic chemicals." Toxicol. Ind. Health 8:239-254.

Figure Legends

Fig. 1. Steps in the purification of *Msp*I-derived subnuclear NPC fractions and NPC constituents from blood leukocytes. Nuclei from blood leukocytes were digested according to previously described procedures to generate 6 subnuclear chromatin NPC fractions (S1, M1, S2, M2, 0.1K and R) that were separated by low ionic strength gel electrophoresis with or without DNase-I treatment. The separated NPC fractions were transferred from the gel by electrobloting to Nytran paper, hybridized with ^{32}P-labeled mycoplasma gene probes and subjected to autoradiography.

Fig. 2. Identification of mycoplasma gene sequences by Gene Tracking in a leukocyte cell sample taken from a subject who has GWI-CFIDS. The figure is an autoradiogram that shows the binding of a radioactive probe against a specific mycoplasma gene DNA sequence in *Mycoplasma fermentans* (incognitus strain) to isolated 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 subject is infected with *M. fermentans* (incognitus strain).