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Chronic Mycoplasmal Infections in Autism Patients

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Abstract

A majority of Autism patients have systemic bacterial, viral and fungal infections that may play an important part in their illnesses. We found that immediate family members of veterans diagnosed with Gulf War Illnesses (GWI) often complain of fatiguing illnesses, and upon analysis they report similar signs and symptoms as their veteran family members, except that their children are often diagnosed with Autism. Since a relatively common finding in GWI patients is a bacterial infection due to *Mycoplasma fermentans*, we examined military families (149 patients: 42 veterans, 40 spouses, 32 other relatives and 35 children with at least one family complaint of illness) selected from a group of 110 veterans with GWI who tested positive (~42%) for mycoplasmal infections. Consistent with previous results, over 80% of GWI patients who were positive for blood mycoplasmal infections had only one *Mycoplasma* species, *M. fermentans*. In healthy control subjects the incidence of mycoplasmal infection was ~8.5% and none were found to have multiple mycoplasmal species ($P<0.001$). In 107 family members of mycoplasma-positive GWI patients there were 57 patients (53%) that had essentially the same signs and symptoms as the veterans and were diagnosed with Chronic Fatigue Syndrome (CFS/ME) and/or Fibromyalgia Syndrome. The majority of children (n=35) in this group were diagnosed with autism. Most of these CFS or Autism patients also had mycoplasmal infections compared to the few non-symptomatic family members ($P<0.001$), and the most common species found was *M. fermentans*. In contrast, in the few non-symptomatic family members that tested mycoplasma-positive, the *Mycoplasma* species were usually different from the species found in the GWI patients. The results suggest that a subset of GWI patients have mycoplasmal infections, and these infections can be transmitted to immediate family members who subsequently display similar signs and symptoms, except for their children who are often diagnosed with Autism. In a separate study in Central California we examined autism patients and also found a high incidence of mycoplasmal infections, but in contrast to the military families a variety of *Mycoplasma* species were detected in Autism patients.

INTRODUCTION

Autism is characterized by inability to communicate, form relationships with others and respond appropriately to the environment. Autism patients do not all share the same signs and symptoms, but they tend to share certain social, communication, motor and sensory problems that affect their behavior in predictable ways. These children often show repetitive behaviors and develop troublesome fixations with specific objects, and they are often painfully sensitive to certain sounds, tastes and smells [1]. These signs and symptoms are thought to be due to abnormalities in brain function or structure. In some patients there are also a number of other less specific chronic signs and symptoms. Among these are fatigue, headaches, gastrointestinal and vision problems and occasional intermittent low-grade fevers and other signs and symptoms.

Although the exact causes of Autism are not known (genetic defects, heavy metal, chemical and biological exposures, etc.) and are probably different in each patient, there may be some similarities in genetic defects and environmental exposures [2, 3] that are important in patient morbidity (sickness) or in illness progression. Other chronic illnesses have some of the same chronic signs and symptoms, suggesting that there may be some overlap in the underlying causes of these conditions or at least in the factors that cause illness or morbidity or illness progression.

The complex signs and symptoms that evolve in many, perhaps even in a majority of chronic illness patients, may be due, in part, to systemic chronic infections (bacteria, viruses, fungi) that can penetrate into the central nervous system (CNS). Such infections often follow acute or chronic heavy metal, chemical, biological (viral, bacterial, fungal infections) or environmental insults or even multiple vaccines that have the potential to suppress the immune system and leave children susceptible to opportunistic infections [2-5]. These illnesses probably evolve slowly over time in a multistep process that may require multiple genetic defects along with multiple toxic exposures.

Chronic infections may be an important element in the development of Autism. Such infections are usually held in check by immune surveillance, but they can take hold and become a problem if they can avoid host immunity and penetrate and hide in various tissues and organs, including cells of the CNS and peripheral nervous system. When such infections occur, they may cause many of the complex signs and symptoms seen in various chronic illnesses [5, 6]. Changes in environmental responses and increased titers to various endogenous viruses as well as bacterial and fungal infections have been commonly seen in chronic illnesses [5, 6]. One type of airborne infection that has received renewed interest of late as an important cause, cofactor or opportunistic infection in various chronic illnesses is represented by relatively primitive classes of bacteria. These microorganisms, principally *Mycoplasmas* and other bacteria (*Chlamydia, Coxiella, Brucella, Borrelia*, etc.), although not as well known as other agents in causing disease, are now considered important emerging pathogens in various chronic diseases where a majority of patients have evidence of these infections in their blood [5, 6].

Autism patients often show their first signs and symptoms after multiple childhood immunizations [2]. In fact, Rimland [2] noted that the sharp rise in Autism only occurred after the multiple vaccine MMR came into widespread use. In the U.S. children typically receive as many as 33 vaccines, a dramatic increase in the use of childhood vaccines over the last few

decades. Such vaccines often contain mercury and other preservatives [3]. Commercial vaccines have also been examined for contaminating microorganisms, and one study found that approximately 6% of commercial vaccines were contaminated with mycoplasmas [6]. Thus we examined the extent of mycoplasmal infections in patients with Autism. We were aided in this examination by data that we already collected on families of Gulf War veterans where there was a high incidence of mycoplasmal infections in their children [8].

METHODS

Patients

Gulf War veterans with GWI and a positive test for mycoplasmal infection and their immediate family members (149 patients: 42 veterans, 40 spouses, 32 other relatives and 35 children) were enrolled in the Gulf War Illnesses study [8]. Seventy age-matched healthy volunteers were recruited and used as control subjects. In the Central California Autism study 18 children diagnosed with Autism were enrolled. All subjects underwent a medical history and routine laboratory tests. If necessary, medical records were also reviewed to determine if patients suffered from organic or psychiatric illnesses that could explain their symptoms [8]. All subjects completed an illness survey questionnaire, which included demographic information, known environmental exposures, dates of illness onset, health status before and immediately after the Gulf War and current health status. We also used an Autism Illness Survey Form developed by the Autism Institute (San Diego, CA). Control subjects had to be free of disease for at least three months prior to data collection.

Blood Collection

Blood was collected in EDTA-containing tubes, immediately brought to ice bath temperature and shipped with wet ice by air courier to the Institute for Molecular Medicine and International Molecular Diagnostics, Inc. for analysis. All blood samples were blinded. Whole blood was used for preparation of DNA using Chelex as previously described [8, 9]. Multiple Mycoplasma tests were performed on all patients and control subjects [8, 9].

Amplification of Gene Sequences by PCR

Amplification of the target gene sequences by Polymerase Chain Reaction (PCR) was accomplished as previously described [8, 9]. Negative and positive controls were present in each experimental run. The amplified samples were separated by agarose gel electrophoresis. After denaturing and neutralization, Southern blotting was performed to confirm the PCR product [8, 9]. Multiple PCR primer sets were used for each species tested to minimize the chance that cross-reacting microorganisms were detected.

Statistics

Subjects' demographic characteristics were assessed using descriptive statistics and students' t-tests (independent samples test, t-test for equality of means, 2-tailed). The 95% confidence interval was chosen. Pearson Chi-Square test was performed to compare prevalence data between patients and control subjects. Illness survey data were statistically analyzed using Spearman Rank correlation and Mann-Whitney tests.

RESULTS

Gulf War Illness Family Study

As found in previous studies [10, 11], veterans of the Gulf War with chronic illnesses (GWI) exhibited multiple signs and symptoms. Upon examination, the signs and symptoms of GWI were indistinguishable from civilian patients diagnosed with CFS/ME, expect for symptomatic children aged 3-12 who were also diagnosed with Autism [8].

Similar to previous studies [10, 11], 45 of 110 GWI patients or ~42% had mycoplasmal infections (Figure 1) , and almost all of these (37 out of 45 or ~82%) were single infections (one species of mycoplasma) [8]. *M. fermentans* was found in ~85% of these single infection cases (Figure 2). When the few multiple infection cases were examined, most were found to have combinations of *M. fermentans* plus either *M. pneumoniae, M. hominis* or *M. genitalium* (Figure 2). In contrast, in healthy control subjects only 6 of 70 subjects (8.5%) were positive for mycoplasmal infections, and all of these were single species infections of various types [8]. Comparing GWI patients and non-symptomatic control subjects, there was a significant difference in the incidence of mycoplasmal infections ($P<0.001$). However, significant differences in infection incidence or species of mycoplasmal infection between male and female GWI patients or control subjects were not seen in these patient groups [8].

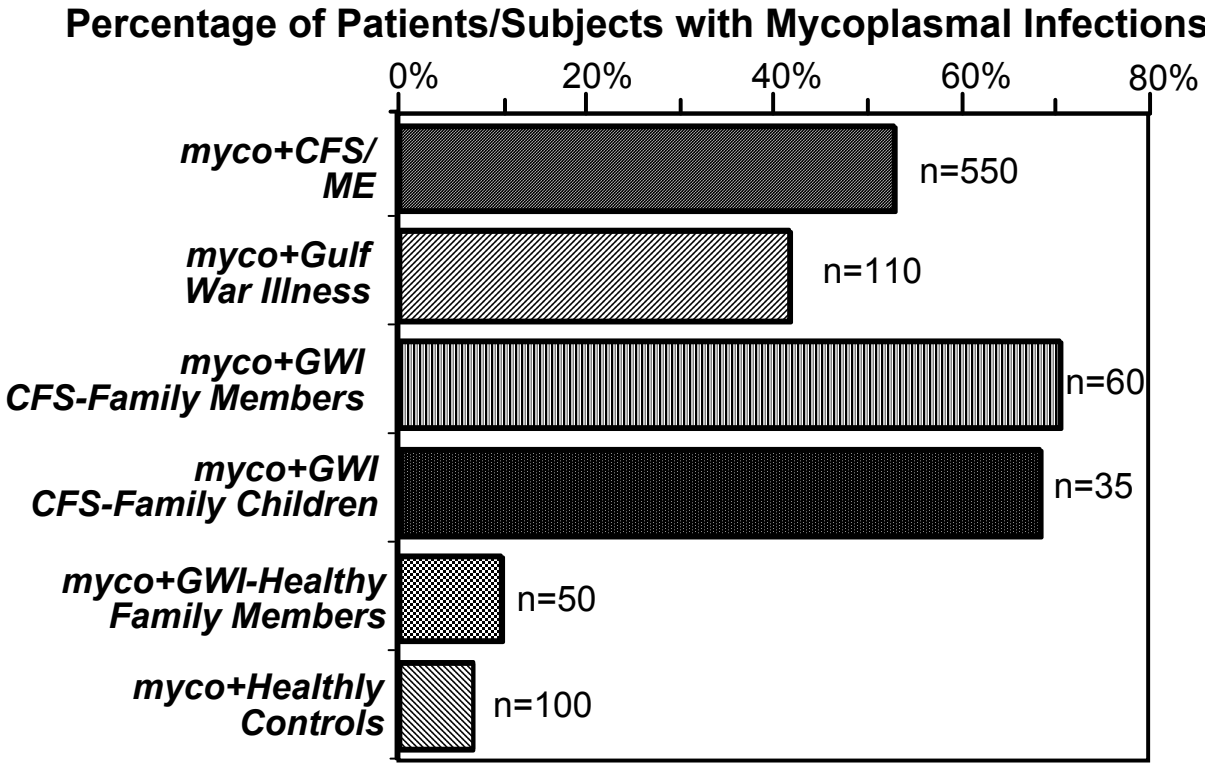

Figure 1. Percent incidence of mycoplasmal infections in family members of veterans with Gulf War Illnesses.

In family members of Gulf War veterans with GWI there was evidence of transmission of the illness. These families were not randomly chosen; they were families in which one or more

veteran members were found to be positive for a mycoplasmal infection and one or more family members reported illnesses. We found that 57 out of 107 (53.2%) of these members from families with one or more Gulf War veteran diagnosed with GWI and with a positive test for a mycoplasmal infection showed symptoms of CFS/ME. Among CFS-symptomatic family members, most (40 out of 57 or 70.2%) had mycoplasmal infections compared to the few non-symptomatic family members who had similar mycoplasmal infections (6 out of 50 or 12%) (Figure 1). When the incidence of mycoplasmal infection was compared within families, the CFS-symptomatic family members were more likely to have mycoplasmal infections compared to non-symptomatic family members ($P<0.001$). Symptomatic children (mostly diagnosed with Autism and other chronic disorders) in these families were also infected with mycoplasmas at high incidence (Figure 1), but this was not seen in aged-matched control subjects (data not shown). Although some non-symptomatic family members did have mycoplasmal infections (6 out of 50 or 12%), this was not significantly different from the incidence of mycoplasmal infections in healthy control subjects (6 out of 70 or 8.5%) (Figure 1).

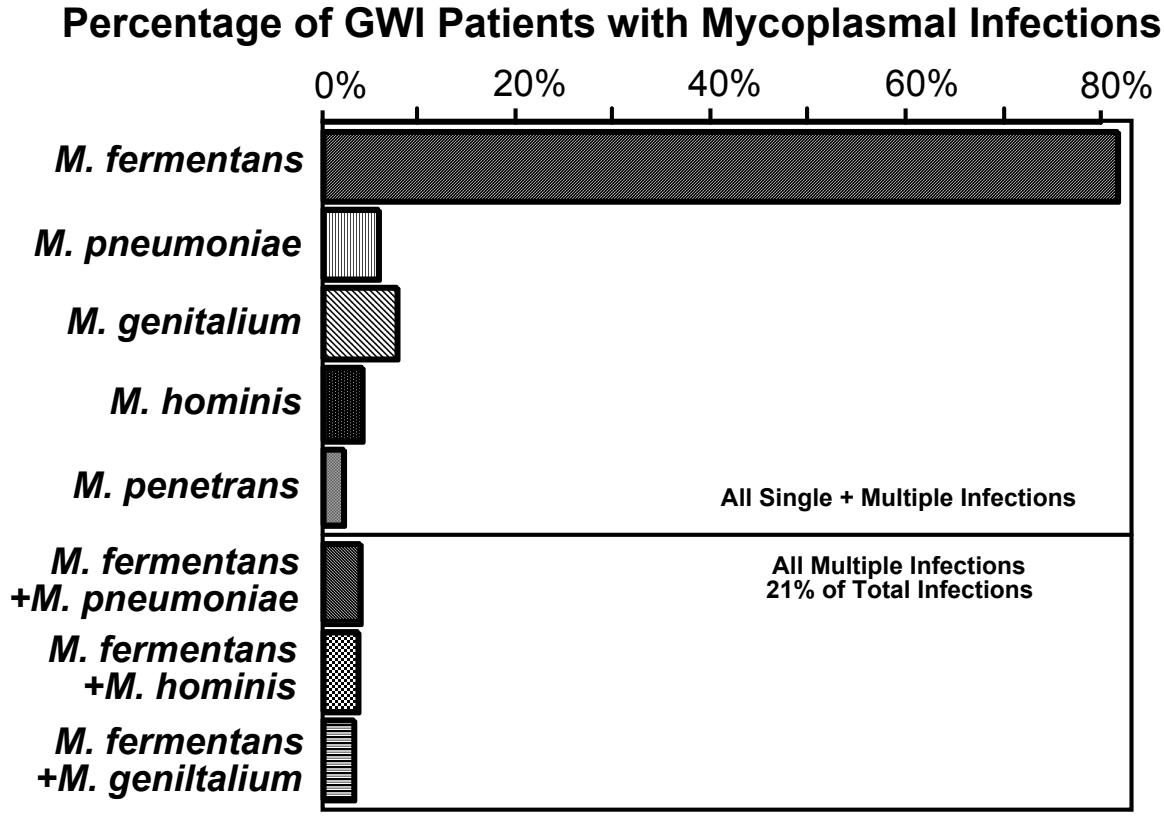

Figure 2. The incidence of various mycoplasma species in Gulf War Illnesses. All cases of multiple mycoplasmal infections were combinations of *M. fermentans*.

The mycoplasma infection types were also similar between GWI patients and their CFS-symptomatic family members. In 45 mycoplasma-positive CFS-symptomatic family members, most (31 out of 40 or 77.5%) had single species infections, similar to the mycoplasma-positive Gulf War veterans (37 out of 45 or 82%). Most mycoplasma-positive GWI patients as well as mycoplasma-positive family members with CFS or children diagnosed with Autism had *M. fermentans* (Figure 3). We did not find differences in the incidence of infection or type of

infections between males and females, children versus adults or spouses versus other family members (data not shown). However, similar to previous reports, the time of onset of CFS illness after the Gulf War tended to be shorter in spouses than other family members, but these differences did not achieve significance [8].

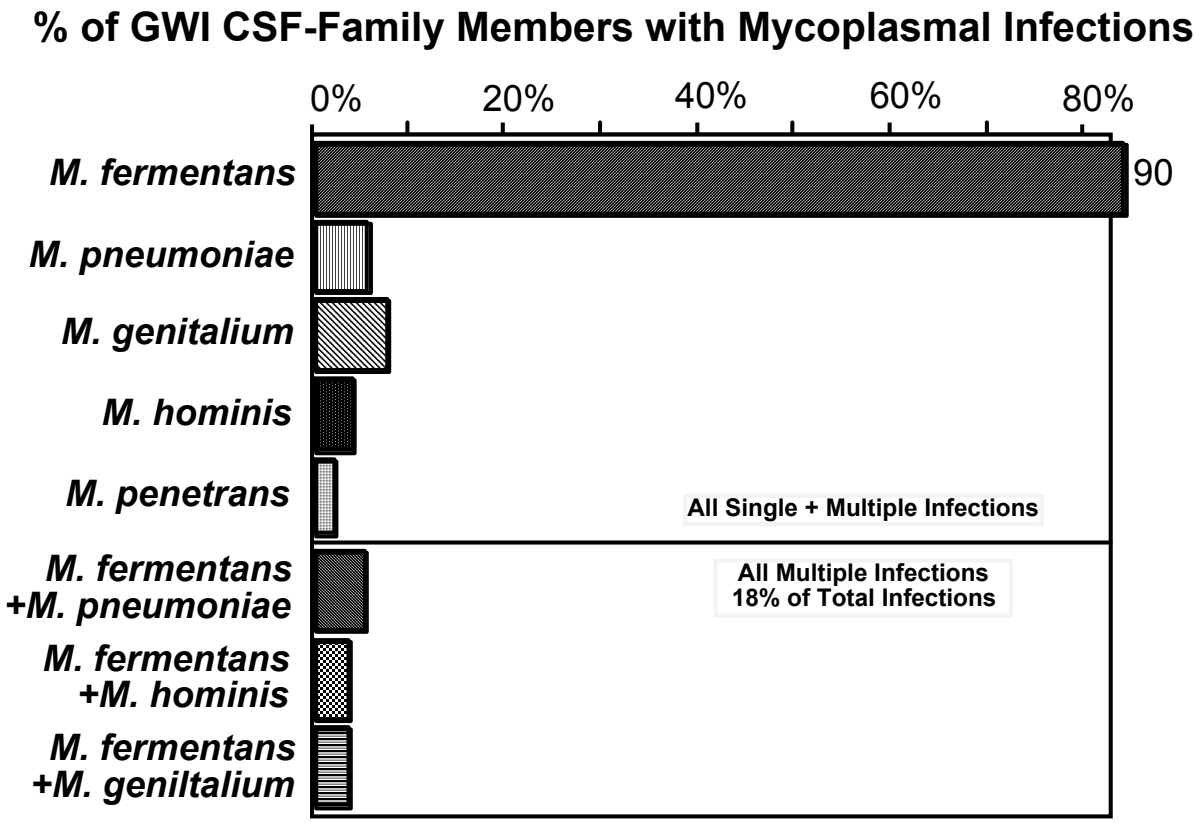

Figure 3. The incidence of various mycoplasma species in family members of veterans with Gulf War Illnesses. All cases of multiple mycoplasmal infections were combinations of *M. fermentans*.

Autism Pilot Study

We next examined a small cohort of Autism patients in Central California. This comprised 18 patients aged 3-11 who were diagnosed with Autism. Most of these children had at least one parent with a chronic illness, and the most common diagnosis of adults or adolescents in the same family was CFS/ME or Fibromyalgia Syndrome. When the Autism patients were examined for mycoplasmal infections, twelve children tested positive (66%) for mycoplasmal infections. However, in contrast to the children of GWI patients who for the most part had only one type of mycoplasmal infection, M. fermentans, the Central California group that tested positive for mycoplasmal infections had a variety of different species of mycoplasmas (Figure 4). We also tested a few siblings without apparent signs and symptoms, and for the most part few had these infections (3 out of 23 subjects or 13%). Similar results were found in the Gulf War veterans' families where 12% of nonsymptomatic family members had mycoplasmal infections [8]. The finding of a variety of different species of mycoplasmas in Autism patients was similar

to the results in a number of studies on CFS/ME and FMS patients where multiple infections of various species of mycoplasmas were commonly found [9].

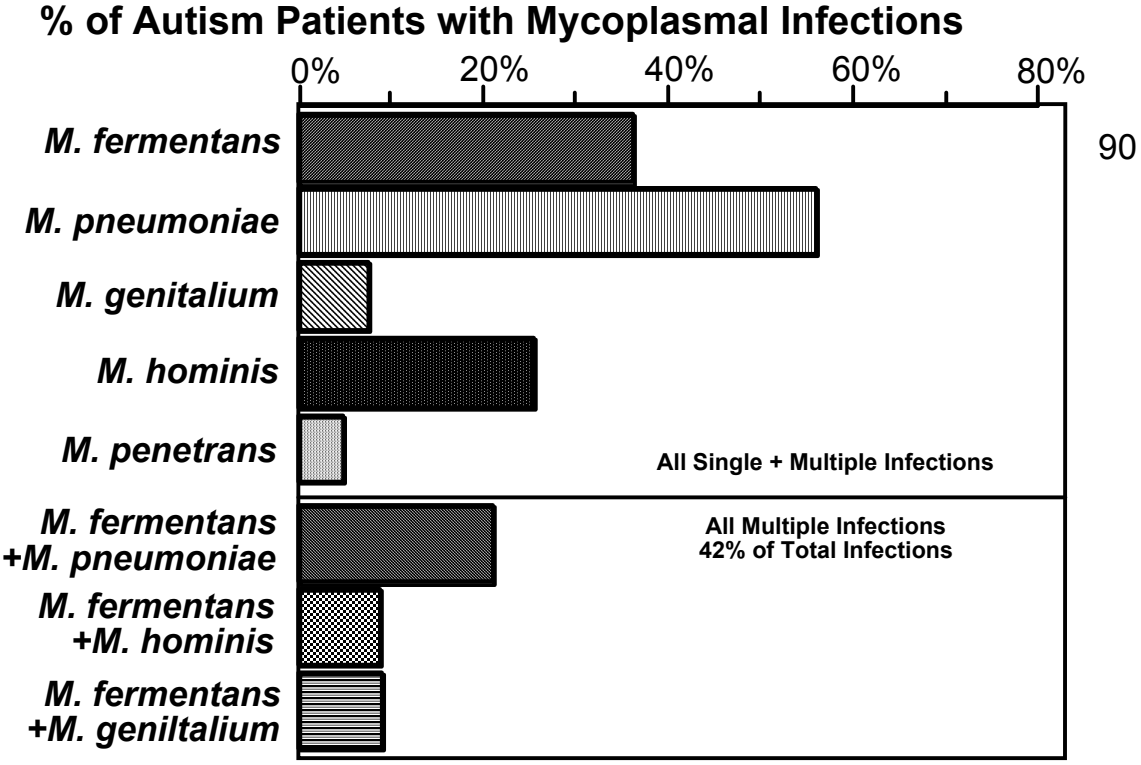

Figure 4. The incidence of various mycoplasma species in patients with Autism from Central California. All cases of multiple mycoplasmal infections were combinations of *M. fermentans*.

DISCUSSION

Although the results presented here document that the chronic infections found in Gulf War veterans with GWI can be found in symptomatic family members, including their children with Autism, we cannot extrapolate our results to the entire GWI patient population or their family members [8]. First, our patient sample was not randomly selected. The presence of a positive mycoplasma test result on a veteran with GWI who reported illness in his/her immediate family formed the criteria for inclusion in the study. Although chronic illnesses in immediate family members were commonly seen in our study, which examined families of mycoplasma-positive GWI patients, these illnesses are expected to be more difficult to find in the general GWI population where chemical, radiological and environmental exposures probably account for the majority of cases. Second, GWI patients and their family members were recruited from veterans groups, word of mouth, physician referrals and the Institute for Molecular Medicine website (www.immed.org); they were not recruited from specific military units. Although some of these patients were examined by physicians at our associated clinics, most were seen by their own private physicians. Fourth, the validity of PCR techniques for *Mycoplasma* species detection has

been questioned. In our studies, however, the sensitivity and specificity of the PCR method for *Mycoplasma* species detection were determined by examining serial dilutions of purified DNA from *M. fermentans, M. pneumoniae, M. hominis* and *M. genitalium* or the microorganisms themselves in blood samples. The primers produced the expected amplification product size in all test species, which was confirmed by hybridization using the appropriate ^{32}P-labeled internal probe. Amounts as low as a few fg of purified DNA were detectable for all species with the specific internal probes. There was no cross-reactivity between the internal probes of one species and the PCR product from another species [12].

Symptomatic family members of GWI patients were diagnosed with CSF/ME or a related fatiguing illness, Fibromyalgia Syndrome (FMS) but their symptomatic children were usually diagnosed with Autism [8]. At least 50-60% of CFS and/or FMS patients are positive for mycoplasmal infections [5, 6, 9, 12-16]. However, in contrast to mycoplasma-positive GWI patients and their mycoplasma-positive family members diagnosed with CFS/ME or Autism, several species of mycoplasmas in addition to *M. fermentans* were found in CSF/ME and FMS patients from non-military families [12-16]. Similarly, we also found various species of mycoplasma in children diagnosed with Autism from Central California. This further supports the hypothesis that mycoplasmal infections were transmitted from GWI patients to immediate family members [8].

There could be different sources of the mycoplasmal infections found in GWI patients [17]. An important possible source for the mycoplasmal infections found in GWI patients is the multiple vaccines that were administered during the time of deployment to the Persian Gulf. A strong association has been found between GWI and the multiple vaccines that were administered during deployment [18-20]. Also, Steele [20] found a three-fold increased incidence of GWI in non-deployed veterans who had been vaccinated in preparation for deployment, compared to non-deployed, non-vaccinated veterans, and Mahan et al. [21] found a two-times higher incidence of GWI signs and symptoms in veterans who recalled receiving anthrax vaccinations versus those who thought they had not. Although the mycoplasmal infections found in GWI patients could have come from several sources, including offensive Biological Warfare attacks [22], we consider the most likely source of the mycoplasmal infections in GWI patients was the multiple vaccines administered during deployment [17]. Indeed, the signs and symptoms that have developed in Armed Forces personnel who recently received the anthrax vaccine are similar to those found in GWI patients. On some military bases this has resulted in chronic illnesses in as many as 7-10% of personnel receiving the vaccine [23]. Undetectable microorganism contaminants in vaccines could have resulted in illness, and this may have been more likely in individuals with compromised immune systems caused by chemical and other exposures [17]. Similarly, the onset of Autism in children from civilian families is also associated with multiple vaccines [2].

Contamination with mycoplasmas has been found in commercial vaccines. In one study 6% of commercial vaccines were found to be contaminated with mycoplasmas [7]. Thus the vaccines used in the Gulf War should be considered as a possible source of the chronic infections found in mycoplasma-positive GWI patients and by airborne transmission in their mycoplasma-positive, CFS-symptomatic family members. And the appearance of mycoplasmal infections in children

diagnosed with Autism from civilian families may eventually be linked to the multiple vaccines received during childhood.

REFERENCES

1. Neuwirth, S. Autism. NIH Publication No. 97-4023, 1997.

2. Rimland, B. The Autism epidemic, vaccinations and mercury. *J. Nut. Environ. Med.* 2000; **10:**261-266.

3. Downing, D. Mercury again. *J. Nut. Environ. Med.* 2000; **10**:267-269.

4. Nicolson GL. Chronic infections as a common etiology for many patients with Chronic Fatigue Syndrome, Fibromyalgia Syndrome and Gulf War Illnesses. *Intern. J. Med.* 1998; **1:**42-46.

5. Nicolson GL, Nasralla M, Hier J, et al. Mycoplasmal infections in chronic illnesses: Fibromyalgia and Chronic Fatigue Syndromes, Gulf War Illness, HIV-AIDS and Rheumatoid Arthritis. *Med. Sentinel* 1999; **4**:172-176.

6. Nicolson GL, Nasralla M, Franco AR, De Meirlier K, Nicolson NL, Ngwenya R, Haier J. Mycoplasmal infections in fatigue illnesses: Chronic Fatigue and Fibromyalgia Syndromes, Gulf War Illness and Rheumatoid Arthritis. *J. Chronic Fatigue Syndr* 2000; **6**(3/4):23-39.

7. Thornton D. A survey of mycoplasma detection in vaccines. *Vaccine* 1986; **4:**237-240.

8. Nicolson, G.L., Nasralla MY, Nicolson NL, Haier J. High prevalence of mycoplasmal infections in symptomatic (Chronic Fatigue Syndrome) family members of mycoplasma-positive Gulf War Illness patients. *J. Chronic Fatigue Syndr.* 2002; **10**:in press.

9. Nasralla M, Haier J, Nicolson GL. Multiple mycoplasmal infections detected in blood of Chronic Fatigue and Fibromyalgia Syndrome patients. *Eur. J. Clin. Microbiol. Infect. Dis.* 1999; **18:**859-865.

10. Nicolson GL, Nicolson NL. Diagnosis and treatment of mycoplasmal infections in Persian Gulf War Illness-CFIDS patients. *Intern. J. Occup. Med. Immunol. Tox*. 1996; **5**:69-78.

11. Nicolson GL, Nicolson NL, Nasralla M. Mycoplasmal infections and Chronic Fatigue Illness (Gulf War Illness) associated with deployment to Operation Desert Storm. *Intern. J. Med.* 1998; **1**:80-92.

12. Nasralla M, Haier J, Nicolson GL. Detection of mycoplasmal infections in blood of 565 chronic illness patients detected by polymerase chain reaction. *Intern J. Med. Biol. Environ.* 2000; **28**(1):15-23.

13. Nicolson GL, Nasralla M, Haier J, et al. Diagnosis and treatment of chronic mycoplasmal infections in Fibromyalgia and Chronic Fatigue Syndromes: relationship to Gulf War Illness. *Biomed. Ther.* 1998; **16**:266-271.

14. Vojdani A, Franco AR. Multiplex PCR for the detection of *Mycoplasma fermentans, M. hominis* and *M. penetrans* in patients with Chronic Fatigue Syndrome, Fibromyalgia, Rheumatoid Arthritis and Gulf War Illness. *J. Chronic Fatigue Syndr.* 1999; 5:187-197.

15. Nicolson GL, Nasralla M, Franco AR, et al. Diagnosis and Integrative Treatment of Intracellular Bacterial Infections in Chronic Fatigue and Fibromyalgia Syndromes, Gulf War Illness, Rheumatoid Arthritis and other Chronic Illnesses. *Clin. Pract. Alt. Med.* 2000; **1**:92-102.

16. Vojdani A, Choppa PC, Tagle C, et al. Detection of *Mycoplasma* genus and *Mycoplasma fermentans* by PCR in patients with Chronic Fatigue Syndrome. *FEMS Immunol. Med. Microbiol.* 1998; 22:355-365.

17. Nicolson GL, Berns P, Nasralla M, et al. Gulf War Illnesses: chemical, radiological and biological exposures resulting in chronic fatiguing illnesses can be identified and treated. *J. Chronic Fatigue Syndr.* 2002; **11**(1):135-156.

18. Unwin C, Blatchley N, Coker W, et al. Health of UK servicemen who served in the Persian Gulf War. *Lancet* 1999; **353**:169-178.

19. Cherry N, Creed F, Silman A, et al. Health and exposures of United Kingdom Gulf war veterans. Part II: The relation of health to exposure. *J. Occup. Environ. Med.* 2001; **58**:299-306.

20. Steele L. Prevalence and patterns of Gulf War Illness in Kansas veterans: association of symptoms with characteristics of person, place and time of military service. *Amer. J. Epidemiol.* 2000; **152:**992-1002.

21. Mahan CM, Kang HK, Ishii EK, et al. Anthrax vaccination and self-reported symptoms, functional status and medical conditions in the national health survey of Gulf War era veterans and their families. Presented to the Conference on Illnesses among Gulf War Veterans: A Decade of Scientific Research. Military and Veterans Health Coordinating Board, Research Working Group. Alexandria, VA: January 24-26, 2001.

22. Nicolson GL, Nicolson NL. Gulf War Illnesses: complex medical, scientific and political paradox. *Med. Conflict Surviv*. 1998; **14:**74-83.

23. Nicolson GL, Nass M, Nicolson NL. The anthrax vaccine controversy. Questions about its efficacy, safety and strategy. *Med. Sentinel* 2000; **5**:97-101.