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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 nfection 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 theirchildren 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 hadto 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].