had antibodies to nuclear envelope antigens [4] while another study found the same ANA antibody rates in both CFS and controls [5]. Recently, investigators reported that antibodies to the human muscarinic cholinergic receptor 1 may provide a biologic explanation for the cognitive impairment observed in people with CFS [6]. This lack of consensus between studies may in large-part reflect recruitment bias associated with studies of persons enrolled from tertiary referral clinics combined with imprecise evaluation of the illness and inadequate or inappropriate control populations.

We had the opportunity to measure the associations of common autoantibodies and autoantibodies to neuronal cell antigens and CFS in two case control studies; one of primary care patients with CFS who were identified by a physician surveillance network; the other a study of people with CFS identified from the community. The physician surveillance study was conducted 1988 through 1993 in Atlanta, Georgia [7] and the community study 1997 through 2000 and identified subjects with CFS from the general population of Wichita, Kansas [8].

Both studies rigorously classified people as CFS and controls in both studies were enrolled to represent the general population and matched to cases by sex, race, and age [9,10]. The hypothesis of the present study is that the appearance of cell-specific autoimmune antibodies may define subsets of CFS and give clues to the etiology and pathogenesis and may help to explain the neurocognitive symptoms experienced by CFS patients. Secondarily, we wished to evaluate the extent to which patients with CFS who were receiving primary medical care treatment for CFS were similar to people with CFS in the community.

Methods

Study Subjects and samples

Both studies adhered to human experimentation guidelines of the U.S. Department of Health and Human Services and the Helsinki Declaration. The Centers for Disease Control and Prevention (CDC) Institutional Review Board approved study protocols. All participants were volunteers who gave informed consent.

Physician surveillance study

Between 1988 and 1993, the CDC conducted a physician surveillance survey for CFS in primary care patients from Reno, Nevada, Wichita, Kansas, Grand Rapids, Michigan, and Atlanta, Georgia [7]. Patients were classified as CFS according to the 1988 case definition [11]. In 1992, we conducted a case control study of CFS patients and controls in Atlanta by recruiting patients from physician surveillance and sex, race, age matched non fatigued controls identified in the general Atlanta population [9,10]. The case control study classified patients as CFS according to the study collected information concerning several risk factors and blood to measure associations between CFS and laboratory markers. The present study used remaining archived serum samples from 22 CFS patients and 34 age and sex matched controls. All CFS patients met criteria of the current CFS research case definition [1]

Population study participants

Between 1997 and 2000, CDC conducted surveillance of CFS in the general population of Wichita, Kansas [8]. Briefly, the study involved random digit dial surveys to identify people with CFS-like illness and clinically evaluated and classified them according to criteria of the 1994 CFS research case definition [1]. Only 16% of those identified with CFS had been diagnosed or treated for CFS by a physician [12]. The present study used archived serum samples from 37 subjects with CFS and a 57 non-fatigued control subjects

Blood samples

Both the physician surveillance and population study collected blood in BD Vacutainer Serum tubes. The samples were shipped by overnight courier to CDC where they were dispensed into 0.5 ml aliquots and stored at -80°C until testing.

Reagents and Assays

Commercially available kits were used for antibodies to ubiquitous nuclear and cellular autoantigens including dsDNA, ssDNA, Sm, U1-RNP, SS-A/Ro, SS-B/La, Scl-70, and Centromere. Immunoassays were purchased from Helix Diagnostics (West Sacramento, CA) and reagents for western blots were purchased from Diagnostic Products Corporation (Los Angeles, CA). Purified Histone H3, and Histone H4 were purchased from Sigma (St. Louis, MO). and used in ELISA assays that were developed at Scripps. Conventional immunofluorescent antinuclear antibodies and rheumatoid factor tests were performed as described previously [13]. Preparations of microtubule-associated protein 2 (MAP2) and neurofilament triplet (NFT) proteins were purchased from Sigma (St. Louis, MO). The commercially available ELISA assays were performed according to the manufacturers instructions. The ELISA and western blot assays for the neuronal antigens were developed at Scripps and were performed as previously described [14].

Statistical Analysis

Because the subjects are derived from studies that are distinct in design and geographic location, each study was analyzed separately. The distribution of autoantibodies between CFS and non-fatigued controls was compared by Fisher exact probability test. To derive an estimate of confidence, stratified groups were compared by the non-parametric chi square test. To determine associations,