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No Findings of Enteroviruses in Swedish Patients with Chronic Fatigue Syndrome

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Enteroviruses have been proposed to cause an immune complex disease in the chronic fatigue syndrome. Altogether 34 patients with the chronic fatigue syndrome, according to criteria of the Centers for Disease Control, USA, were studied evenly over the seasons for the possible presence of a chronic enterovirus infection. In 11 patients, 1–5 faecal samples were collected at about 6 month intervals for virus isolation before and after acid treatment, followed by ultracentrifugation at pH 3 to dissolve possible enterovirus–antibody complexes. Another 14 faecal samples were subjected to routine virus isolation alone. Seven pairs of serum–cerebrospinal fluid samples were analysed for cross-reactive IgG antibody activity to enteroviruses. In 29 patients a muscle biopsy was collected for enterovirus polymerase chain reaction (PCR). We were unable to identify enteroviruses in any of these samples by any of these techniques. Our study does not confirm evidence for persistent enterovirus infection in the chronic fatigue syndrome.

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INTRODUCTION

The chronic fatigue syndrome (1) every year disables numerous previously healthy people from work (1). In an interesting article by Yousef et al. (2), enteroviruses have been proposed as the cause of an immune complex disease in a number of these patients. Furthermore, enterovirus genomes have been found in the muscle (3) by enterovirus polymerase chain reaction (PCR). Also, enterovirus PCR products were identified in serum and/or leucocytes in patients with the chronic fatigue syndrome in a similar proportion to or higher than that in patients, acutely ill in what was clinically suggestive of an enterovirus illness (4, 5). It is necessary to confirm these findings in other laboratories and patients for validation.

MATERIALS AND METHODS

Altogether 34 patients (22 women and 12 men) were included; all fulfilled the criteria of the Centers for Disease Control (CDC), Atlanta, USA, for the chronic fatigue syndrome from 1988 by Holmes et al. (6). The mean age at the onset of illness was 35 years (range 19–54 years, median 34 years). All 34 patients had an acute flu-like episode or a history of another infectious disease at the onset of their disease. 50% (17/34) had pronounced muscle symptoms, including postexercise fatigue. 53% (18/34) showed pronounced neuropsychiatric symptoms. 12 of these patients had symptoms of both types. The mean duration of illness at diagnosis was 2.7 years (range 0.5–7 years, median 2.0 years).

The patients were studied evenly over the seasons from February 1989 to July 1993 to avoid accumulation of sampling during the enterovirus season. In all patients, 1–5 faecal samples were collected at about 6 months interval for virus isolation before and after acid treatment. The shortest interval was 1 month and the longest 8 months. Another 14 faecal samples were subjected to routine virus isolation alone (7). Paired serum and cerebrospinal fluid (CSF) samples were collected from 7 patients, all of whom showed pronounced symptoms from the central nervous system

(CNS). In 29 patients a muscle biopsy was collected during the period from December 1992 to June 1993. Although all these patients had some symptoms from their muscles, 15 had predominantly CNS symptoms, whereas 14 had predominantly muscle symptoms. Six of these patients had been studied also by analysis from both faecal and serum–CSF samples, due to the presence of symptoms from both locations.

The techniques used for the faecal samples were as described (2). A 20% faecal sample solution (7) was subjected to virus isolation before and after ultracentrifugation at pH 3, which was followed by resuspension of the pellet and neutralization of the pH (2). The cells used for isolation were green monkey kidney cells (GMK), rhabdomyosarcoma (Rd) cells and HeLa cells (7). A blind passage was performed after 14 days for another week. The faecal samples were also analysed by direct electron microscopy analysis and by an investigation after airfuge centrifugation, which enables concentration of viral particles of a similar size to enteroviruses (8).

The 14 serum–CSF samples, 7 pairs, were analysed for cross-reactive IgG antibody activity to enteroviruses (9) in a similar way as for intrathecal antibody synthesis in suspected cases of herpes simplex encephalitis (10). Thus, serum and CSF were diluted 4-fold in 0.75% bovine serum albumin and Tween 20 enzyme-linked immunosorbent assay (ELISA) buffer. The serum was diluted from 1:50 and CSF from 1:12.5. The 96-well plates were coated with heated antigens of echovirus 30, coxsackievirus type B5 and echovirus type 9 (9). Control wells were coated with morbilli virus antigen (10). The ratio of titres towards morbilli virus antigen versus enterovirus antigen between serum–CSF pairs may allow the determination of brain barrier damage versus intracellular production of enterovirus antibodies or the absence of enterovirus antibodies in CSF.

A semi-nested PCR protocol for enterovirus RNA (11) was applied to the muscle biopsies, using the guanidine method of RNA extraction. The first primer sets were composed of nucleotides 445–470 and 580–599 (corrected from Glimåker et al. (11) with permission from Bo Johansson) at the 5' non-coding region, followed in the second PCR by nucleotides 445–470 and 544–564, respectively, giving the amplified product a size of 120 base pairs. The nucleotide numbers were in alignment with coxsackievirus type B3 (GenBank). Heart muscle and lung tissue from a fatal case of enterovirus infection served as positive controls.

Table I. Search for enteroviruses by different techniques in patients with the chronic fatigue syndrome

	No. of patients	No. of samples
Virus identification by:		
Isolation in faeces	0/12	0/46
After acid treatment and ultracentrifugation	0/11	0/32
Electron microscopy	0/12	0/46
Serology in CSF-serum pairs	0/7	0/7
PCR in muscle biopsies	0/29	0/29
Total	0/34	0/82

This study was approved by the Ethics Committee of the Karolinska Institutet, Stockholm, Sweden.

RESULTS

The results were as seen in Table I. In addition, there were findings by electron microscopy of small round unstructured viruses in 3/12 patients in 4/46 samples. This is in agreement with "background" findings in our laboratory, without apparent association with disease (unpublished observations, K-OH and AE). Also, while no enterovirus antibodies were found in the CSF, enterovirus antibodies were detected in the serum of 5/7 subjects. This antibody activity was detected at a dilution range of 1:200 to 1:3200. Both the frequency and the titers of enterovirus antibodies corresponded to normal findings.

DISCUSSION

We were unable to repeat the findings of enterovirus isolates identified by virus isolation after ultracentrifugation at low pH to dissolve antibody-virus immune complexes, as described by Yousef et al. (2). Furthermore, a search for enterovirus by electron microscopy, by looking for intrathecally produced enterovirus IgG antibodies, and investigation of enterovirus RNA in muscle biopsies by PCR, failed to give an indication of the presence of enterovirus in any of these sties.

It may be mentioned that the acid dissociation attempt to dissolve enterovirus antibody complexes was technically laborious and tedious to perform. It is questionable whether 1 ultracentrifugation at low pH may be sufficient to both resolve complexes and prevent reassociation when the pellet is resolved at neutral pH. Perhaps another second or third such treatment would be necessary. As it was not mentioned in the paper to have been done more than once, we performed the experiment as published (2). Also, it is quite common that enteroviruses may be excreted in stools from patients with enterovirus infection weeks or even months after the appearance of neutralizing antibodies (7), and then both virus and possible complexes seem to disappear. In a persistent RNA virus infection, defective virus particles would be expected to appear which not necessarily

would be infectious. The identification of genome parts such as PCR products or antigens is therefore intriguing. Still, the association of enterovirus with the chronic fatigue syndrome requires that results are in agreement between different laboratories in different epidemiological settings in order to be convincing.

The negative finding of the presence of enteroviral RNA sequences in muscle biopsies in the present study is in agreement with a larger study on 121 patients with the chronic fatigue syndrome in whom 101 biopsies showed a similar frequency of enteroviral RNA to those of other patients (12). Two publications from a virus laboratory in Glasgow, Scotland, in 1995 (4, 5) point to the possibility that enterovirus RNA may be present in the serum in a proportion of patients with the chronic fatigue syndrome. Until there is a well-evaluated clinically useful test for enterovirus RNA detection in serum in patients with a proven enterovirus infection such as virus isolation from stools or the CSF, it is difficult to evaluate these results, especially in the absence of concordant results from different laboratories.

The aspiration of finding enterovirus in these patients was primarily related to epidemiological implications (14). The findings by Yousef et al. (2), as well as those of enteroviral RNA in muscle biopsies (3, 13), raised the hope of identifying an etiological agent to this devastating disease among working age patients. Our study lends support to the large survey on the absence of evidence for persistent enterovirus infections in chronic human disease (15).

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