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Postviral fatigue syndrome: persistence of enterovirus RNA in muscle and elevated creatine kinase

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Summary

Enterovirus-specific probes have been prepared by reverse transcription of conserved sequences in purified Coxsackie B2 virus genomic RNA and molecular cloning techniques. These probes were used in quantitative slot blot hybridizations to test for the presence of enterovirus-specific RNA in skeletal muscle biopsy specimens from 96 patients who had suffered from the postviral fatigue syndrome myalgic encephalomyelitis for up to 20 years. Biopsy specimens from 20 patients were positive for the presence of virus-specific RNA with hybridization signals more than three standard deviations greater than the mean of the normal muscle controls. Biopsies from the remaining 76 patients were indistinguishable from the controls.

These data show that enterovirus RNA is present in skeletal muscle of some patients with postviral fatigue syndrome up to 20 years after onset of disease and suggest that a persistent virus infection has an aetiological role.

Introduction

The genus Enterovirus of the family Picornaviridae consists of more than 70 serotypes including the 6 members of the group B Coxsackieviruses and the 3 serotypes of polioviruses. The enterovirus group has been associated with a number of diseases ranging from aseptic meningitis and minor upper respiratory tract infections to paralytic poliomyelitis, myocarditis, hepatitis and fulminating multisystem infection of the neonate¹. Recently there has been interest in a link between persistent enterovirus infection and the postviral fatigue syndrome myalgic encephalomyelitis^{2,3}. This syndrome is characterized by many symptoms, but particularly by severe exhaustion and muscle

fatiguability following an apparent viral infection. More than half of the patients also suffer myalgia and many complain of dysequilibria, sleep disturbances and psychogenic symptoms³. The syndrome has been observed worldwide for the past 50 years, in epidemics and as sporadic cases. The observation that the syndrome is diagnosed particularly frequently in medical or hospital service staff and the failure to demonstrate objective clinical signs led to the proposition of a hysterical origin⁴.

Improved criteria for diagnosis and the employment of additional laboratory technologies have revealed organic lesions⁵. Electromyographical (EMG) investigations have shown that many patients give abnormal responses, particularly in single fibre EMG where some patients exhibit prolonged jitter values⁶ a feature previously observed in patients with acute viral infections⁷. Nuclear magnetic resonance spectroscopy (NMR) has shown that muscle of some of these patients undergoes premature intracellular acidosis during exercise and has a prolonged recovery period⁸, indicating dysfunction of respiratory metabolism. Investigations of lymphocyte populations of patients ill for up to 20 years reveal that the helper/suppressor T-cell ratio is low compared with the normal population⁵, a characteristic previously observed in virus-infected subjects⁹. Serological studies have shown that about 30% of postviral fatigue patients are positive for Coxsackie B virus-specific IgM compared with 9% of normal controls in the same study and that these responses persisted in some patients for at least one year¹⁰.

Persistent enterovirus infection, particularly with the group B Coxsackieviruses, has been implicated in a number of disorders by such serological investigations. However, attempts to isolate virus or demonstrate the presence of virus-specific antigens in the affected tissue have generally been unsuccessful in chronic disorders. Despite these observations, we have demonstrated previously that enterovirus RNA (probably Coxsackie B virus) is present in the affected tissue in inflammatory and chronic myopathies of cardiac or skeletal muscle by molecular hybridization of an enterovirus-specific cDNA probe to RNA isolated from biopsy specimens^{11,12}. We now report analogous data demonstrating the persistence of virus RNA in skeletal muscle biopsies from patients suffering from postviral fatigue syndrome (PFS).

Methods

Muscle biopsy specimens from the quadriceps of 96 patients diagnosed clinically as suffering from PFS, together with muscle from 4 normal controls, were investigated. Total nucleic acids were isolated from portions of these specimens (<10 mg), blotted onto nylon membranes and hybridized with an enterovirus

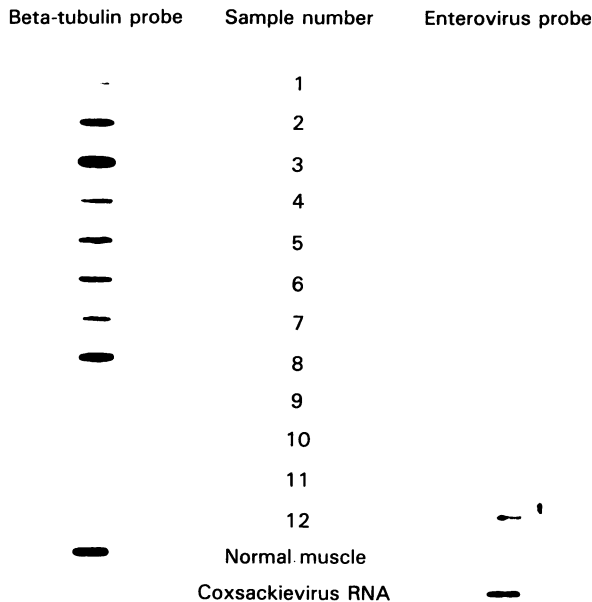


Figure 1. Representative autoradiographs after hybridization of enterovirus and beta-tubulin-specific probes to RNA isolated from skeletal muscle biopsy specimens.

specific probe (derived by reverse transcription and molecular cloning of the genome of a Coxsackie B2 virus) as described previously^{11,12}. Briefly, the tissue was digested overnight in detergent/protease in the presence of placental ribonuclease inhibitor, and nucleic acid purified by phenol/chloroform extraction and ethanol precipitation. After recovery by centrifugation the nucleic acids were redissolved in $6\times$ SSC, 7.5% formaldehyde ($1\times$ SSC=0.15 M sodium chloride, 0.015 M trisodium citrate, pH 7.0) and the RNA selectively denatured by heating at 65°C for 10 min. The RNA was immobilized on duplicate nylon filters using a slot blot apparatus, in parallel with purified Coxsackie B2 virus genomic RNA as a positive control. The filters were probed with either a 0.85 kb virus-specific cDNA or a beta-tubulin cDNA¹³, labelled with ³²P by random oligomeric primer extension¹⁴. The virus-specific probe is complementary to sequences derived from near the 3' end of the virus genome which encodes the RNA-dependent RNA polymerase. This sequence is highly conserved amongst enterovirus serotypes (except Hepatitis A virus) and this probe is, therefore, enterovirus group-specific¹⁵. The beta-tubulin probe was employed to quantify the amount of RNA immobilized from each sample. After hybridization and washing at high stringency, the filters were autoradiographed using presensitized X-ray film. The autoradiographic development was quantified by scanning densitometry and the strength of the signal generated with the virus-specific probe was expressed as a ratio to the strength of the signal with the beta-tubulin probe.

Results

RNA isolated from 96 skeletal muscle needle biopsy specimens was immobilized on duplicate nylon membranes together with positive and negative controls. One filter was hybridized with the enterovirus-specific probe and the other with the beta-tubulin probe. Figure 1 shows a representative autoradiograph after hybridization to RNA from a proportion of the specimens studied. The hybridization signal produced by each sample with either probe was

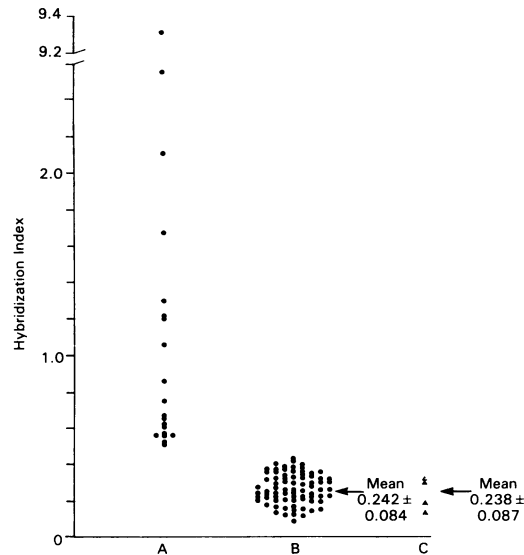


Figure 2. Detection of enterovirus-specific RNA in skeletal muscle biopsy specimens. Groups A and B are patients with postviral fatigue syndrome. Group A is positive and group B negative for virus RNA. Group C are normal controls.

determined by scanning densitometry of suitably exposed autoradiographs. The ratio of the signal produced by the virus-specific probe to that of the control probe was determined and termed hybridization index (HI).

The relative signal produced by each of the specimens is shown in Figure 2. The 96 specimens from the PFS patients clearly fall into 2 groups. Seventy-six of these give low, background values (Figure 2, group B: mean HI=0.242 \pm 0.084) and are statistically indistinguishable from the normal muscle controls (Figure 2, group C: mean HI=0.238 \pm 0.087). The remaining 20 specimens from patients suffering from PFS were positive for the presence of enterovirus-specific RNA (Figure 2, group A) giving hybridization indices more than three standard deviations greater than the mean of the controls or the remainder of the PFS group. In analogous experiments we have probed more than fifty samples of normal human muscle. These have been consistently negative for enterovirus RNA.

Other studies performed included muscle NMR, single fibre EMG, serum creatine kinase (CK) assay, Coxsackie B virus-specific IgM and neutralizing antibody determinations and histopathological assessment of muscle biopsy specimens. The results of these for the 20 patients positive for enterovirus-specific RNA are summarized in Table 1. None of the characteristics shown in Table 1 distinguished the virus-probe positive group from the remainder except for the values of serum CK. Of the 96 PFS patients studied 11 had elevated CK values, and of these 9 were found to be positive for enterovirus RNA.

Discussion

The nature and aetiology of postviral fatigue syndrome have been contentious for many years. Recently, the application of a range of techniques has facilitated a clearer description of the underlying lesion. Objective techniques such as NMR and single fibre EMG have revealed abnormalities of muscle biochemistry and physiology which may account for the clinical presentation of muscle weakness and extreme fatigability in many of these patients.

Table 1. Patient details and results of laboratory studies of patients with muscle biopsy samples positive for enterovirus RNA.

Patient	Sex	Age	Duration of disease (years)	Muscle biopsy findings●	CK■ (units/ml)	Coxsackie antibody titres		Single fibre EMG
						IgM	N/A	
1	F	49	6/12	Positive	400	B4	nd	Negative
2	F	18	3	Positive	310	Negative	nd	Negative
3	F	33	2/12	Positive	300	B2,3,5	B3(1024)	Positive
4	F	34	5	Negative	270	Negative	B4(1024)	Negative
5	F	44	4	Negative	265	Negative	Negative	Positive
6	F	50	>10	Positive	260	Negative	Negative	Positive
7	F	41	1	Negative	210	Negative	B2(256)	Positive
8	M	37	20	Negative	180	Negative	Negative	Positive
9	M	52	15	Positive	170	nd	Negative	Negative
10	F	21	3/12	Positive	<150	nd	Negative	Negative
11	F	18	1	Negative	<150	Negative	nd	Positive
12	F	39	1	Negative	<150	Negative	B4(512)	Negative
13	M	39	4.5	Positive	<150	nd	nd	Positive
14	F	60	2	Positive	<150	nd	B2(256)	Negative
15	F	69	20	Negative	<150	Negative	Negative	Positive
16	F	40	2	Positive	<150	B4	nd	Positive
17	F	40	18/12	Positive	<150	nd	nd	Negative
18	F	48	2.5/12	Positive	<150	Negative	nd	Negative
19	M	49	1	Positive	<150	Negative	nd	Negative
20	M	24	7/12	Negative	<150	Negative	B4(512)	Negative

●Non-specific changes; ■ Normal range for CK = <150 units/ml; nd, not done.

Serological investigations have implicated Coxsackie B viruses, amongst others, as aetiological agents. However, conventional virological techniques have generally failed to demonstrate the presence of virus in these patients. This situation is reminiscent of other chronic muscle diseases where Coxsackie B viruses have been implicated by retrospective serology but attempts to isolate infectious virus or to detect virus-specific antigens are consistently unsuccessful.

Using techniques by which we have previously demonstrated the presence of enterovirus RNA in biopsy specimens from patients with myocarditis, dilated cardiomyopathy or inflammatory diseases of skeletal muscle^{11,12} we have now demonstrated that 20 of 96 PFS patients studied had enterovirus RNA in muscle biopsy specimens. This figure may be an underestimate of the occurrence of enterovirus-specific RNA within affected muscle because of the sampling errors arising from the study of a small portion of the single biopsy specimen taken from each patient. Our studies of enterovirus infection of cardiac muscle in patients with myocarditis or dilated cardiomyopathy or of skeletal muscle in patients with myositis, have revealed that the focal nature of the infection correlates with the pathology observed in these disorders. It seems likely that the study of multiple tissue samples will provide an increased figure for virus involvement.

The duration of disease amongst the enterovirus positive group ranged from 2 months to 20 years. Assuming that virus infection was the trigger for the onset of disease, it is clear that enteroviruses are capable of persisting in the affected tissue for many years. The IgM results suggest that only in patients with recent onset disease is there a continuing immune response to the normal viral antigens. The proportion of patients positive for virus RNA described here with significant neutralizing titres (55%) is similar to the previously reported frequency in PFS patients³ (46%). In addition, some patients

may be infected with another enterovirus serotype detected by molecular hybridization with the group-specific probe but not in a Coxsackie B virus-specific neutralization test.

Histological and histochemical investigations of muscle biopsy specimens from PFS patients have revealed a number of non-specific abnormalities, primarily, scattered atrophic fibres, type II fibre hypertrophy and occasional single fibre necrosis. The proportion of patients positive for virus-specific RNA and exhibiting muscle abnormalities (60%) was not significantly different from those who were negative for virus RNA.

The most striking feature of the enterovirus-positive group was the number with mildly raised serum creatine kinase (CK) activities. This enzyme has been used as a marker of muscle damage and large increases in serum CK concentration have been reported in patients with various myopathies. Moderately increased CK values (up to about 2 times normal values) have been reported previously in patients with PFS¹⁶. Of the 96 patients studied here 11 had consistently elevated CK values and of those, 9 were positive for virus RNA. Therefore, of those in whom a clear demonstration of continuing muscle damage could be made, over 80% had a persisting enterovirus infection of their muscle.

One patient, positive for enterovirus RNA and who had suffered from the disease for more than 10 years, had abnormal histology and single fibre EMG, elevated serum CK and also showed NMR abnormalities of the type described previously⁸. Chronic viral illnesses are often characterized by the negation of some non-housekeeping functions within cells such that the cells survive but with an altered metabolism¹⁷. As enteroviruses clearly are capable of persistent infection of muscle, it is possible that interference with muscle metabolic functions results from their presence and explains the abnormal findings of NMR and EMG.

The hybridization studies reported here represent the first direct demonstration of the presence of enterovirus RNA within the skeletal muscle of patients with PFS and provides further evidence of the organic nature of this syndrome. However, it is likely that although enteroviruses are major aetiological agents of PFS, other viruses, particularly Epstein-Barr virus¹⁸, may induce the syndrome. We suggest that this disease is a chronic metabolic myopathy induced by persistent virus infection.

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Coxsackie B viruses and myalgic encephalomyelitis

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Summary

Data collected over the past 6 years suggest that Coxsackie B viruses (CBV) play an important role in myalgic encephalomyelitis (ME). Since psychological upset is a feature of this illness, 247 patients, recently admitted to a psychiatric hospital, were tested for neutralizing antibodies to CBV. A total of 12.5% had significantly raised CBV titres compared with 4-5% of 'well' control groups; the percentage positive was greatest (21%) in those aged 30-39 years.

During 1985 and 1986 sera from 290 adults with ME were tested using the newly developed CBV IgM ELISA test; 37% were CBV IgM positive compared with 9% of 500 'well' adult controls. Forty-seven

children, with ME were similarly tested during this period; 38% were positive, implying recent or active CBV infection. The combined use of this ELISA test and the virus probe techniques now available should further help to elucidate the exact role of CBV in this disabling illness.

Introduction

Outbreaks and sporadic cases of myalgic encephalomyelitis (ME) have been reported from many parts of the world during the past 50 years. Various terms used to describe this bizarre illness have included epidemic neuromyasthenia, Iceland Disease and Royal Free Disease named after a large outbreak in that London Hospital in 1955. Currently the term ME is regarded as that which best encompasses the multiple symptomatology associated with this illness¹. Women are more often affected than men and a curious susceptibility is shown by nursing and medical staff. The afflicted patients have a wide variety of complaints but these always include muscle pain, extreme fatigue on exertion and psychological upset. Although most give a history of a preceding viral-like illness investigators have usually failed to identify a virus and the absence of objective abnormalities has led many to propose that the illness is psychogenic.

An outbreak of ME occurred in the West of Scotland in 1980/81. The only positive virological finding was