Persistent virus infection of muscle in postviral fatigue syndrome

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Nucleic acid was extracted from muscle biopsy samples from a series of highly selected patients suffering from chronic muscle fatiguability following a viral infection (Postviral Fatigue Syndrome: PVFS). Samples were examined for the presence of enteroviral RNA sequences or Epstein-Barr (EBV) virus DNA sequences by molecular hybridisation as these two agents have been implicated by retrospective serology in the aetiology of PVFS. We found enteroviral RNA in 24% of biopsy samples and EBV DNA in a further 9% of biopsy samples: no biopsy was positive for both enteroviral RNA and EBV DNA. In addition, in the case of enteroviruses we found that the persisting virus is defective in control of RNA replication as both strands of enteroviral RNA are present in similar amounts: this is unlike the asymmetric synthesis of genomic RNA seen in a productive, cytolytic enterovirus infection. The implications of these data in relation to mechanisms of viral persistence and muscle dysfunction are discussed.

We use the term Postviral Fatigue Syndrome (PVFS) to describe an illness whose major symptoms are excessive fatiguability of muscle after moderate exercise and a prolonged recovery period: these symptoms must have been present for at least 6 months and many patients can date their fatiguability to an episode of demon-

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strated or presumed viral illness. Additional symptoms such as myalgia and fasciculation are sometimes present and some patients, with good premorbid personality, complain of psycho-genic symptoms such as sleep disturbance, dysphasia, lack of concentration and emotional lability. The wider clinical syndrome of chronic fatigue is defined elsewhere in this issue (Behan and Bakheit): PVFS patients form a subset of the total number of patients who present with chronic fatigue. Diagnosis is essentially by exclusion of other causes of chronic fatigue such as endocrine disease, malignancy, substance abuse or psychiatric illness.

The association of chronic fatigue with viral infection arose because a number of patients identified the onset of their fatiguability as a 'flu-like illness from which they did not fully recover' and serological evidence from these patients suggested an association with infection by either an enterovirus or Epstein-Barr virus. The evidence for a direct involvement in pathogenesis by persistent infection of muscle in PVFS is examined for each of these.

**ENTEROVIRUS INFECTION AND PVFS**

Enteroviruses are members of the family Picornaviridae and consist of about 70 serotypes, including Coxsackie A and B viruses, ECHO viruses and the polioviruses. Although frequent agents of trivial or even subclinical enteric illness, this group of viruses has been associated with more serious disease including upper respiratory tract infection, aseptic meningitis, paralytic poliomyelitis, congestive heart failure and fulminating multisystem infection of the neonate.\(^1\) These viruses appear to have particular tropisms for muscle and nervous tissue.

The enteroviruses are small icosahedral particles, approximately 27 nm in diameter and have a single-stranded RNA genome about 7.4 kilobases (kb) in length. The virus genome has mRNA function, i.e. is of positive polarity, has a poly A tract of 50–150 nucleotides at the 3' end and can act as an experimental template to prime cell-free protein synthesis. After infection of cultured cells in vitro cellular protein synthesis is selectively inhibited by cleavage of one of the proteins involved in cap-dependent initiation of protein synthesis (p220, one component of eIF-4F).\(^2\) Initiation of translation of virus-specific proteins takes place by a cap-independent mechanism and so is unaffected by p220 function. The viral genomic RNA encodes a single open reading frame (ORF) which is translated to yield a 260 kD precursor polyprotein: this
is cleaved subsequently by virus-encoded, site-specific proteases to generate the individual viral proteins, both enzymatic and structural. The virus genome is replicated by a virus-coded, RNA-dependent RNA polymerase via a complementary negative sense or template strand: virus RNA synthesis is asymmetric and, in productive infection, (+) genomic RNA predominates over (−) template RNA by about 100:1. Following assembly of the virus structural proteins and encapsidation of a single copy of the genomic RNA in each progeny virus, the cell is lysed and many infectious particles are released.

Initially, the link between PVFS and enterovirus infection was provided by epidemiological studies. During a poliomyelitis outbreak in Los Angeles in 1934, many medical staff developed PVFS-like symptoms. A similar epidemic occurred in Akureyri, Iceland in 1948 which subsequently protected the population from poliovirus infection, suggesting that they had been infected with a related virus at that time. Two more recent serological studies of patients with PVFS suggest a role for enteroviruses, particularly the Coxsackie B viruses, as pathogenetic agents. Elevated neutralizing antibody titres against Coxsackie B viruses were detected in 50% of PVFS patients compared with 17% of healthy controls. More significantly, Coxsackie B virus-specific IgM (indicating recent or persistent infection) was detected in 31% of patients compared with 9% of controls: virus-specific IgM responses were detected in sequential sera from some PVFS patients over one year or longer, suggesting persistent virus infection.

The detection of the enterovirus structural protein VP1 in immune complexes from the serum of PVFS patients provides further evidence for the aetiological role of enteroviruses. This viral antigen was detected in 44 of 87 (51%) of patients compared with none of 36 controls: of those positive, 39 (89%) were still positive 4 months later. After acid dissociation of neutralising antibodies, infectious virus was isolated from the stools of 15 of 74 patients but none of 28 controls: the same serotype of virus was isolated from 5 of the 15 patients when retested one year later.

While these data implicate persistent enterovirus infection in the pathogenesis of PVFS, the fact the virus persists in the gut of a proportion of patients may account for the serological observations but does not explain the reported aberrations of muscle metabolism and electromyography. Attempts to demonstrate either infectious virus or virus-specific antigens in muscle samples from PVFS patients had been consistently unsuccessful.
situation is comparable to enterovirus-related heart muscle disease (myocarditis or dilated cardiomyopathy) in which virus could not be isolated after the early stages of disease, but serological data suggested persisting enterovirus infection. The use of molecular hybridisation probes capable of detecting enterovirus RNA in endomyocardial tissue samples demonstrated that, in a proportion of patients, virus RNA could be detected in the affected tissue for years after the onset of symptoms. Enterovirus-specific probes were used similarly to investigate muscle biopsy samples to determine whether an analogous situation occurred in PFS patients.

The strategy used was quantitative slot blot hybridisation with a cloned cDNA probe. This was prepared by reverse transcription of purified Coxsackie B2 virus genome RNA by priming with an oligo (dT) sequence complementary to the 3' polyadenylated terminus of the genome. Complementary DNA synthesised in this way was ligated into a cloning vector and propagated by conventional molecular biology techniques. Poliovirus type 1 was the first enterovirus genome to be cloned and sequenced but several other serotypes have been partially or completely cloned since. Hybridisation to RNA immobilised on solid supports shows that the 5' and 3' non-translated regions and sequences encoding non-structural virus proteins of the virus such as the RNA-dependent RNA polymerase, are highly conserved between members of the enterovirus sub-groups. In practice, probes corresponding to the P1 region, encoding the virus-structural proteins are essentially serotype-specific, whereas probes from other regions are enterovirus group-specific. The nucleotide sequences of several serotypes of enterovirus have been established and have confirmed the homologies predicted by hybridisation. The cloned cDNA probe used in our studies was complementary to nucleotide numbers approximately 5900–6900 of Coxsackie B2 virus genome. This corresponds to the sequence encoding the first two-thirds of the RNA-dependent RNA polymerase gene which is highly conserved between enteroviral serotypes. The resulting probe was enterovirus-group specific, as confirmed by hybridisation to RNA extracted from cells infected with various enteroviral serotypes.

Cloned probes were labelled by random hexanucleotide primer extension: random primers are annealed to a denatured (i.e. single stranded) DNA sequence and the complementary strand synthesised using the Klenow (large) fragment of DNA polymerase
The newly synthesised strand is labelled by addition of a radio-labelled deoxyribonucleotide to the reaction.

Slot blot hybridisation with the enterovirus group-specific probe was used to investigate whether enterovirus RNA could be detected in muscle from patients with PVFS. RNA was isolated by proteinase/detergent digestion in the presence of human placental ribonuclease inhibitor, and subsequent ethanol precipitation, from muscle biopsy samples from quadriceps of PVFS patients or from control tissue. RNA was blotted onto duplicate nitrocellulose or nylon membrane filters. One filter was hybridised with $10^6$ CPM of $[^{32}\text{P}]$-radiolabelled enterovirus group-specific cDNA probe at a specific activity of between 1 and $3 \times 10^9$ DPM/µg; the other was hybridised with a similarly labelled control probe (7B6), to quantitate the total RNA immobilised from each sample. After hybridisation, the filters were washed to high stringency, autoradiographed with pre-sensitised X-ray film and the autoradiographic development quantitated by scanning densitometry. The signal generated with the virus-specific probe was expressed as a ratio to the signal with the control probe (Hybridisation Index: HI): this gives a measure of the relative amount of enterovirus RNA compared to the amount of cellular RNA in any sample. The mean and standard deviation of the HI values of the negative controls, included on all blots, was calculated and compared with the results for the clinical samples. Samples with a value of HI exceeding the mean of the negative controls by more than three standard deviations are considered positive for persistence of enterovirus RNA ($P < 0.0015$; Fig. 1).

To date, we have studied muscle biopsy samples from a total of 140 PVFS patients of which 34 (24%) were positive by molecular hybridisation for the presence of enterovirus RNA. This is a highly significant finding ($P < 0.00001$) as enterovirus RNA was not detected in any of 152 control samples of human muscle (108 normal biopsy samples, taken to exclude various conditions; 28 pathological biopsy samples including 24 Inclusion Body Myositis; 2 samples obtained from orthopaedic surgery and samples from 14 post mortem procedures). Additionally this figure is likely to be an underestimate of the occurrence of enterovirus RNA in muscle of PVFS patients because of sampling errors arising from the study of only a portion of a single muscle biopsy sample from each patient. Our slot blot and in situ hybridisation studies of multiple tissue samples from enterovirus-induced heart muscle disease have revealed that persistent enterovirus infection in
Fig. 1 Detection of enterovirus RNA in skeletal muscle needle biopsy specimens. Groups A and B are samples from patients with postviral fatigue syndrome and samples in group C are from controls. The hybridisation index (HI) is the ratio for each sample of the autoradiographic signal (quantitated by scanning densitometry) with the virus-specific probe to that of the control probe. Samples in the shaded area (groups B and C) are negative for enterovirus RNA (less than 3 × SD greater than the mean HI of control group) and the remainder, group A, are positive.
chronic disease is focal and involves only a small proportion of cardiac myocytes. Analagously, multiple samples of muscle from each PVFS patient would be required to provide a more accurate figure for the frequency of virus involvement.

There was no significant difference in histological or histochemical investigations of muscle biopsy samples between the patient groups positive or negative for enterovirus RNA. A number of non-specific changes, primarily scattered atrophic fibres, type II fibre hypertrophy and occasional single fibre necrosis compatible with the observations of mildly elevated serum CK were seen in some patients.

In one particular series of 96 PVFS patients, 20 (21%) were found to have biopsy samples positive for enterovirus RNA. The duration of disease among the enterovirus positive group ranged from 2 months to 20 years (Table 1) and indicates that enteroviruses are capable of persisting in muscle for many years. Coxsackie B virus-specific IgM assays suggest that only in patients with recent onset of symptoms is there a continuing humoral immune response against normal viral antigens. However, some patients may be infected with a different enterovirus serotype, detected by molecular hybridisation with the group-specific probe but not in a Coxsackie B virus-specific neutralisation antibody or IgM assay. There was a correlation between mildly but consistently elevated serum creatine kinase (CK) concentration and the detection of enterovirus RNA in this series of PVFS patients. This enzyme is a marker of muscle damage and large elevations in serum CK concentration have been reported in patients with various myopathies, including polymyositis. CK concentrations of up to 2 times normal levels have been reported in PVFS patients. Of the 20 patients with muscle samples positive for enterovirus RNA in this study, 9 had consistently elevated CK; in comparison, only 2 of the 76 enterovirus negative patients had similarly elevated serum CK. Therefore, over 80% of the patients in whom continuing muscle damage was indicated by elevated serum CK had a persisting enterovirus infection of muscle. Nine of the 20 patients whose biopsy samples were positive for enterovirus RNA showed abnormally prolonged jitter on examination by single-fibre electromyography. One patient, positive for enterovirus RNA and who had symptoms including muscle fatiguability for more than 10 years, had abnormal histology, exhibited prolonged jitter on a single-fibre EMG, had elevated serum CK and also showed
Table 1  Patient details and laboratory investigations of patients with muscle biopsy samples positive for enterovirus RNA

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<th>Sex</th>
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<th>Coxsackie B antibody titre</th>
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Nuclear Magnetic Resonance (NMR) spectroscopy abnormalities indicative of altered muscle metabolism.

We have used the same enterovirus group-specific hybridisation probes to investigate muscle biopsy samples from 82 cases of histologically proven inflammatory muscle disease. Enterovirus RNA was detected in biopsies from 9 of 46 (20%) cases of adult polymyositis and 14 of 36 (39%) cases of juvenile dermatomyositis, compared to none of the same control population described above. As our PVFS patients date their muscle fatiguability to a prior ‘viral’ illness, this suggests that chronic muscle fatiguability associated with persistent enterovirus infection may be a sequel of a previous enteroviral myositis.

Detection of enterovirus RNA sequences in muscle biopsy samples from cases of inflammatory or chronic muscle disease is clearly a pathological situation as virus RNA was not found in any of a large number of normal or pathologically irrelevant muscle biopsy samples.

PERSISTENT ENTEROVIRUS INFECTION OF MUSCLE IS ASSOCIATED WITH DEFECTIVE VIRUS MUTANTS

The cDNA probe was cloned into a riboprobe vector to study the association between enterovirus persistence in muscle and PVFS. The cloned cDNA sequence was sub-cloned downstream of a promoter for the RNA polymerase encoded by phage SP6. A cRNA probe (riboprobe) was synthesised from this linearised construct using a DNA-dependent RNA polymerase. In the present study, we cloned cDNA in both orientations so that probes complementary to either the sense (genomic or positive) strand or the anti-sense (template or negative) strand could be synthesised independently. These cRNA probes can be used selectively to detect either strand of virus RNA: this allows the relative abundance of the sense strand and the anti-sense strands of enteroviral RNA to be determined in quantitative slot blots. Replication of the enterovirus genome occurs via an anti-sense (template or negative) strand synthesised by the virus-coded RNA-dependent RNA polymerase. In productive, cytolytic infection, this strand normally acts as a template for the preferential synthesis of the sense (genomic or positive) strand which is either translated by cellular ribosomes to yield virus-specific proteins or is packaged into the virus capsid and eventually released from the cell.
We have used cRNA probes to characterise cytolytic infection of cells cultured in vitro. The results show that in LLCMK2 cells synthesis of sense (+) strand predominates over anti-sense (−) by at least 100:13: this confirms previously published data.28 We observed a similar preponderance of positive, genomic strand enteroviral RNA in muscle from CD-1 mice 7 days after intraperitoneal inoculation with Coxsackie B1 (Tuscon) virus. This model of polymyositis30 shows that infectious virus can be isolated for up to 2 weeks post infection: fibre necrosis and infiltration by inflammatory cells indicates active virus replication.

We used these single-stranded riboprobes to study enteroviral RNA isolated from muscle biopsy samples from patients with PVFS. In contrast to productive cytolytic infection and inflammatory muscle disease, samples from all PVFS cases which were positive for enterovirus RNA contained similar amounts of the sense and anti-sense strands.3 Representative data are shown in Figure 2: the control probe is cRNA complementary to the cell-cycle independent mRNA species 7B6.24 These data suggest that persistence of enterovirus RNA in muscle is associated with the emergence of virus mutants defective in the control of virus genomic RNA replication. The molecular basis of this defect is not yet known, but could result from mutations in sequences encoding the RNA-dependent RNA polymerase or in the 3' end of the template strand, where the putative RNA polymerase binding site for the production of positive strand RNA is situated. Single stranded RNA viruses rapidly accumulate such sequence mutations, introduced by the RNA-dependent RNA-polymerase as, after synthesis, there is no complementary sequence against which to repair mis-matches.

EPSTEIN-BARR VIRUS AND OTHER HERPES VIRUSES

The human herpes viruses, including Epstein-Barr virus and Zoster varicella (chicken pox) virus, have also been implicated in the pathogenesis of PVFS. Epstein-Barr virus (EBV) is a ubiquitous pathogen with which most of the population becomes infected sub-clinically during childhood.31 If primary infection occurs during adolescence, infectious mononucleosis (glandular fever) usually results and may last for several years.32 Zoster varicella virus infection leads to the vesicular skin lesions characteristic of chicken pox and may then establish a latent infection of dorsal ganglia: reactivation cause shingles.33
Fig. 2 Characterisation of defective enterovirus mutants persisting in muscle biopsy samples from PVFS patients. RNA extracted from muscle samples, blotted in triplicate and hybridised with single stranded riboprobes specific for (a) 7B6 control; (b) positive genomic strand; or (c) negative template strand of enteroviral RNA. Only the probe complementary to the positive strand detected purified CVB2 genomic RNA. Samples 1, 2, 5, 7, 8, 14 and 15 are positive for enterovirus RNA: in all cases hybridisation signals with riboprobes complementary to either positive or negative strands of enterovirus RNA are approximately equal.
The genome of EBV is a 170 kb double-stranded DNA molecule which has been cloned and sequenced.\textsuperscript{34} Only B-lymphocytes and epithelial cells of primates have been shown to express specific cell-surface receptors for EBV.\textsuperscript{31,35} Infection of lymphocytes results in B-cell differentiation, clonal activation and immunoregulatory disturbances.\textsuperscript{33}

Several studies of patients with symptoms similar to those of PVFS found serological evidence of persistent E-B virus infection, irrespective of a clinical history of infectious mononucleosis.\textsuperscript{36,37} There was no obvious clinical feature which distinguished these patients from those diagnosed as having PVFS due to enterovirus infection. One PVFS patient shown by NMR spectroscopy to have abnormal respiratory metabolism of muscle had developed symptoms of chronic muscle fatiguability after an episode of chicken-pox.\textsuperscript{9}

The detection of DNA viruses such as EBV can be performed by DNA-DNA hybridisation using cloned virus DNA fragments. One problem has been that certain regions of human herpes virus genomes have homology to cellular DNA sequences\textsuperscript{38} and so can give rise to spurious hybridisation signals. The use of cloned DNA fragments from unique regions of the virus genome overcomes this problem. An EBV probe which gave minimal cross-hybridisation and represents coding sequences likely to be conserved between isolates was selected for the present study. The gene probe used was a 3.4 kb Rsal fragment, subcloned, using BamHI linkers, into a pUC9 vector from the 5.1 kb EBV BamHI K restriction fragment. This non-repetitive sequence encodes part of the EBV nuclear antigen 1\textsuperscript{39} (EBNA-1). Some EBV gene probes show cross hybridisation with human DNA: this is not the case with the EBNA 1 probe described here.\textsuperscript{40} Antibodies to EBNA-1, as well as other EBV-specific antigens suggestive of chronic infection have been described in patients with PVFS.\textsuperscript{36,37}

DNA was isolated from additional portions of muscle biopsy samples of 72 patients with PVFS, which were a subset of the patients already studied for involvement of enteroviruses, plus 4 further patients and blotted onto duplicate nitrocellulose membranes.\textsuperscript{40} One filter was hybridised with the EBV-specific probe and the second with a control probe\textsuperscript{41} (γ actin cDNA): after autoradiography and scanning densitometry, the results were expressed as a ratio of the signal with the virus probe to the signal with the control probe as previously. As before, the hybridisation signals from the samples from PVFS patients fell into 2 groups.
Of the test samples 68 were statistically indistinguishable from the negative control group of 48 samples of normal muscle (Fig. 3: groups A and B): however, 8 samples of muscle from PVFS patients gave positive signals, exceeding the mean plus $3 \times SD$ of negative controls ($P = <0.02$). All 8 patients with muscle biopsy

Fig. 3 Detection of Epstein-Barr virus DNA in skeletal muscle biopsy specimens. Groups B and C are samples from patients with postviral fatigue syndrome. Samples in group A are from normal or pathologically irrelevant controls. Samples in groups A and B are negative for E-B virus DNA (less than $3 \times SD$ more than the mean HI of control group) and the remainder, group C, are positive.
samples positive for EBV DNA had typical Postviral Fatigue Syndrome and had serological evidence of EBV infection (Table 2). The duration of disease ranged between 4 months and 12 years. Six of the eight had non-specific abnormalities of muscle histology of the type described above. Six of the eight were tested by single fibre EMG and five showed abnormally prolonged jitter. Two of the eight patients positive for EBV-DNA in muscle were examined by NMR spectroscopy: one was apparently normal while the other showed an abnormally low intracellular pH at rest and abnormal changes in the concentration of intracellular muscle phosphates on recovery after exercise.

By comparison of the hybridisation signal from the E-B virus-specific probe with that of the control probe it was estimated that EBV nuclear antigen-specific sequences were present at between 5 and 50 copies per cell equivalent. Conventional microscopic examination of muscle samples did not reveal a lymphocytic infiltrate, suggesting that the EBV sequences detected were present in muscle fibres and not in lymphocytes. However, while EBV is a B-lymphotrophic virus of primates, fusion of lymphocytes to other cell types is known, for example to epithelial cells in naso-pharangeal carcinoma.\(^{42,43}\) This may be a mechanism which EBV can enter many cell types lacking specific receptors, possibly including myocytes. All biopsies from the PVFS patients in this study were reported negative for the presence of an inflammatory infiltrate. However, it is possible that small numbers of EBV transformed B-lymphocytes are present in the samples and if EBV DNA is present in sufficiently high copy number, these could produce a detectable EBV-specific hybridization signal. Whether the E-B virus DNA sequences actually detected are present in myocytes or in a small number of infiltrating B-lymphocytes is not known as the \textit{in situ} hybridisation data are not available.

To date, we have studied muscle biopsy samples from a total of 86 PVFS patients for EBV sequences and 8 (9\%) were positive. No muscle biopsy sample was positive for both EBV and enterovirus genomic sequences.

**PERSISTENT VIRUS INFECTION OF MUSCLE AND PATHOGENESIS IN POSTVIRAL FATIGUE SYNDROME**

The studies described demonstrate that a proportion of patients with PVFS have a persistent virus infection of muscle for several
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<td>M</td>
<td>12</td>
<td>VCA 1/64</td>
<td>Non-specific changes</td>
<td>Abnormal: prolonged jitter</td>
</tr>
</tbody>
</table>

EBNA = Epstein-Barr virus nuclear antigen
EA = Epstein-Barr virus early antigen
VCA = Epstein-Barr virus capsid antigen
nd = Not done
years after the onset of symptoms. While several studies have demonstrated that the incidence of antibodies against enteroviruses or various Epstein-Barr virus antigens is greater in patients with chronic fatigue than in controls, the prevalence of infection by these agents in the general population makes the data difficult to interpret. In general, infectious virus cannot be isolated nor virus-specific antigens detected by immunocytochemistry in tissue samples from this chronic, non-inflammatory disease. However, molecular hybridization under stringent conditions with enterovirus group-specific probes or non-cross-reacting EBV-specific probes unequivocally demonstrates persistence of virus genetic material in samples of the affected tissue. In the case of chronic enterovirus infection of muscle which does not induce an inflammatory response, we have found consistently that the persisting enterovirus is a mutant defective in the control of viral genomic RNA replication. These probably do not synthesize and process the full range of virus gene products, do not assemble new infectious progeny virus particles, induce cell lysis or attract a cellular immune response. In addition to the remarkably high statistical significance of the detection of enterovirus RNA sequences in muscle biopsies from PVFS patients compared with controls, these observations greatly strengthen the case for an aetiological role of enterovirus in the pathogenesis of chronic muscle fatiguability. This is similar to our observations of the persistence of such replication defective enterovirus mutants in the development of dilated cardiomyopathy.

The case for persistent EBV infection of muscle as a cause of chronic fatiguability is more difficult to sustain. EBV is a ubiquitous infectious agent with an inherent mechanism of latency and a tropism for B-lymphocytes rather than myocytes. While it seems likely that active EBV infection may result in chronic fatigue, it is less obvious than the case with enterovirus infection that this results from muscle dysfunction. Unlike the situation with enteroviruses, no in situ hybridization data exists to demonstrate that the EBV specific DNA sequences we detected in muscle biopsy samples from cases of PVFS reside in myocytes rather than in a small number of infiltrating B-lymphocytes.

The only evidence available so far relating to pathological changes in muscle comes from single-fibre EMG and from NMR spectroscopy studies which have indicated defects in muscle metabolism in some patients. Although the mechanism of such changes are unknown, the perturbation of normal cellular func-
tions by persistent virus infection has been described. For example lymphotrophic choriomeningitis virus infection of mouse results in molecular dysfunction of pituitary, T-helper, thymus follicular and pancreatic β-cells: Coxsackie B virus infection of mouse reduces insulin expression in pancreatic β cells.

We have proposed previously that PVFS is functionally an acquired metabolic myopathy induced by persistent virus infection. This proposal would account for the symptoms of muscle fatiguability and myalgia and for the prolonged recovery time after exercise experienced by most of our PVFS patients. The neurological or psychogenic symptoms are more difficult to relate to persistent virus infection. In some patients, direct infection of the central nervous system may occur since some enteroviruses such as Coxsackie A, ECHO and poliovirus serotypes are also associated with neurological disease. Another possibility is that, in response to virus infection, immune mediators such as interferons or interleukins are released resulting in the further symptoms experienced by some PVFS patients.

Our data from heart muscle disease indicate that dilated cardiomyopathy is a sequel to an enterovirus-induced inflammatory disease (myocarditis). By analogy, we suggest here that PVFS is a sequel to a transient viral myositis. While enteroviruses and, possibly less frequently, EBV are probably aetiologic, it seems possible that a range of agents capable of establishing persistent infection of muscle may induce this syndrome.

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