Documented involvement of viruses in ME/CFS

Margaret Williams **30th December 2009**

For decades it has been known and shown that viruses play a role in ME/CFS. Now there is evidence of a direct association with a gamma retrovirus – XMRV -- that disables the immune system in ME/CFS, thus allowing numerous latent viruses to re-activate, which could result in the protean symptomatology.

As Professor Nancy Klimas said in her November 2009 lecture at the University of Miami: "***We've always thought something like that has to go on in (ME)CFS because you all have some neuro-inflammation. Your brain has a low grade level of inflammation. And you have some inflammation in the tissues that make hormones, particularly in the hypothalamic-pituitary-axis. And this is a virus that infects that type of tissue…"*** (see below).

Latent viruses that have been particularly studied in relation to ME/CFS include Coxsackie B virus (CBV), Epstein Barr virus (EBV) and human herpes virus-6 (HHV-6), and illustrations are provided below.

However, advised by psychiatrists of the Wessely School, in the UK the NICE Guideline of 2007 recommends limited serology testing for certain viruses only, **which excludes testing for Coxsackie B virus, for which there is the most evidence** (testing for Epstein Barr Virus, a particular interest of Professor Peter White is, however, permitted).

Given that a classified synonym for ME/CFS is "post-viral fatigue syndrome" (ICD-10 G93.3) and given that, like the MRC PACE Trial, the NICE Guideline purports to apply to people with "CFS/ME", it is striking that the Guideline states on page 141: "***Serological testing should not be carried out unless the history is indicative of an infection***".

It is notable that the PACE Trial Investigators did not include virological testing of participants in their trial that is based on their theory that patients with "CFS/ME" are merely deconditioned, so it needs to be ascertained what, exactly, do the Wessely School psychiatrists understand the term "post-viral" to mean if not a history indicative of an infection?

The following are illustrations of viral involvement in ME/CFS:

1954

Describing an outbreak of infection of the central nervous system complicated by intense myalgia in late summer 1952 affecting nurses at the Middlesex Hospital, London, the author (ED Acheson, who later became UK Chief Medical Officer) reported the clinical features to be severe muscular pain affecting the back, limbs, abdomen and chest, with evidence of mild involvement of the central nervous system, diarrhoea, vomiting, respiratory distress, paresis and brain stem involvement that included nystagmus, double vision and difficulty in swallowing; additionally, bladder symptoms occurred in more than half the patients. Acheson highlighted this small outbreak because of the similarity to atypical poliomyelitis (ED Acheson. Lancet: Nov 20th 1954:1044-1048). The label of "atypical poliomyelitis" was originally given to ME (The Disease of a Thousand Names. David S Bell. Pollard Publications, Lyndonville, New York, 1991). **Many patients today experience exactly the symptoms described by Acheson, but such symptoms are dismissed by the Wessely School as somatisation and as hypervigilance to normal bodily sensations.**

1955

Acheson described and compared the outbreak at the Royal Free in 1955 with the outbreak at The Middlesex in 1952, noting the relatively prolonged active course of the disease, marked muscular pain and spasm, involvement of the lymph nodes, liver and spleen, tenderness under the costal margins, and ulcers in the

mouth, all of which – if looked for and if not dismissed as somatising -- are still to be found in "pure" ME today (ED Acheson. Lancet: Aug 20th 1955:394-395).

1959

In his detailed review of numerous outbreaks of Benign Myalgic Encephalomyelitis from 1934, Acheson described the common characteristics of the disease and clinical picture, which included agonising muscular pain, headache, nausea, sensory disturbances, stiffness of the neck and back, dizziness, muscular twitching, tremor and in-coordination, localised muscular weakness, emotional lability, problems with memory and concentration, hyperacusis, somnolence and insomnia, with relapses being almost inevitable, together with variability of symptoms. Signs included hepatic enlargement, lymphadenopathy and evidence of CNS involvement, nystagmus being "*almost invariable"* in some of the outbreaks. The question of hysteria was addressed and discounted: "*Final points against mass hysteria as a major factor in the syndrome are the consistency of the course of the illness and the similarities in the symptoms…The disorder is not a manifestation of mass hysteria"* and Acheson specifically warned that the diagnosis of ME should be reserved for those with (virally induced) evidence of CNS damage: ***"If not, the syndrome will become a convenient dumping ground for non-specific illnesses characterised by fluctuating aches and pains, fatigue and depression***", exactly the situation that exists in the UK 50 years after Acheson's prophecy (ED Acheson. American Journal of Medicine, April 1959:569-595).

1978

*"The clinical picture was variable both in the time pattern of its progression and the severity of the symptoms…It became clear early on in the outbreak that there was organic involvement of the central nervous system (and) there was objective evidence of involvement of the central nervous system…The most characteristic symptom was the prolonged painful muscle spasms…Bladder dysfunction occurred in more than 25% of all the patients…**Case to case contact between patients and their relatives also occurred**…McEvedy and Beard's conclusions* (of mass hysteria) *ignore the objective findings of the staff of the hospital of fever, lymphadenopathy, cranial nerve palsies and abnormal signs in the limbs…Objective evidence of brain stem and spinal cord involvement was observed"* (Nigel D Compston. Postgraduate Medical Journal 1978:54:722-724).

1983

"Virological studies revealed that 76% of the patients with suspected myalgic encephalomyelitis had elevated Coxsackie B neutralising titres (and symptoms included) malaise, exhaustion on physical or mental effort, chest pain, palpitations, tachycardia, polyarthralgia, muscle pains, back pain, true vertigo, dizziness, tinnitus, nausea, diarrhoea, abdominal cramps, epigastric pain, headaches, paraesthesiae, dysuria)….The group described here are patients who have had this miserable illness. Most have lost many weeks of employment or the enjoyment of their family (and) marriages have been threatened…" (BD Keighley, EJ Bell. JRCP 1983:33:339-341).

1985

"…from an immunological point of view, patients with chronic active EBV infection appear 'frozen' in a state typically found only briefly during convalescence from acute EBV infection" (G Tosato, S Straus et al. The Journal of Immunology 1985:134:5:3082-3088. Note that "CFS" was then thought to be caused by EBV).

1985

"*Epstein-Barr virus infection may have induced or augmented an immunoregulatory disorder that persisted in these patients" (*Stephen E Straus et al. Ann Intern Med. 1985:102:7-16).

1985

*"The clinical, pathological, electrophysiological, immunological and virological abnormalities in 50 patients with the postviral fatigue syndrome are recorded. These findings confirm the organic nature of the disease (and) suggest that it is associated with disordered regulation of the immune system and persistent viral infection" (*PO Behan, WMH Behan, EJ Bell. Journal of Infection 1985:10:211-222.

1987

*"Ninety percent of the patients tested had antibodies to Epstein-Barr virus and 45% tested had antibodies to cytomegalovirus…if this fatigue syndrome is triggered by an infectious agent, an abnormal immune response may be involved" (*TJ Marrie et al. Clinical Ecology 1987:V:1:5-10).

1987

*"Recently associations have been found between Coxsackie B infection and a more chronic multisystem illness. A similar illness…has been referred to as… myalgic encephalomyelitis…140 patients presenting with symptoms suggesting a postviral syndrome were entered into the study…Coxsackie B antibody levels were estimated in 100 control patients…All the Coxsackie B virus antibody tests were performed blind…Of the 140 ill patients, 46% were found to be Coxsackie B virus antibody positive…This study has confirmed our earlier finding that there is a group of symptoms with evidence of Coxsackie B infection. We have also shown that clinical improvement is slow and recovery does not correlate with a fall in Coxsackie B virus antibody titre" (*BD Calder et al. JRCGP 1987:37:11-14).

1987

*"The illness has an acute onset after a variety of infections and then enters a chronic phase characterised by fatigue and numerous other symptoms….Other findings include a sleep disorder, mild immunodeficiency, slightly low complement, anti-DNA antibodies and elevated synthetase which is an interferon-associated enzyme commonly increased in viral infections" (*Irving E Salit. Clinical Ecology 1987:V:3:103-107).

1988

"These results show that chronic infection with enteroviruses occurs in many PVFS (post-viral fatigue syndrome, a classified synonym for ME/CFS) *patients and that detection of enterovirus antigen in the serum is a sensitive and satisfactory method for investigating infection in these patients….Several studies have suggested that infection with enteroviruses is causally related to PVFS…The association of detectable IgM complexes and VP1 antigen in the serum of PVFS patients in our study was high…This suggests that enterovirus infection plays an important role in the aetiology of PVFS" (*GE Yousef, EJ Bell, JF Mowbray et al. Lancet January 23rd 1988:146-150).

1988

"*Myalgic encephalomyelitis was thought for some time to be produced by a less virulent strain of poliovirus…**chronic, persistent viruses may often be reactivated during this illness…once reactivated, do these viruses then go on to produce many of the symptoms of the disease? And what reactivates these endogenous viruses? Could it be environmental toxins? Could it be infection with other, exogenous lymphotropic viruses?***" (Anthony L Komaroff. Journal of Virological Methods. 1988:21:3-10).

(In the light of the discovery in 2009 of the XMRV retrovirus – see below -- this paper by Professor Komaroff 21 years in advance of that discovery showed remarkable prescience).

1988

*"Postviral fatigue syndrome / myalgic encephalomyelitis… has attracted increasing attention during the last five years…Its distinguishing characteristic is severe muscle fatiguability made worse by exercise…The chief organ affected is skeletal muscle, and the severe fatiguability, with or without myalgia, is the main symptom. The results of biochemical, electrophysiological and pathological studies support the view that muscle metabolism is disturbed, but there is no doubt that other systems, such as nervous, cardiovascular and immune are also affected…Recognition of the large number of patients affected…indicates that a review of this intriguing disorder is merited….**The true syndrome is always associated with an infection**…Viral infections in muscle can indeed be associated with a variety of enzyme abnormalities…(Electrophysiological results) are important in showing the organic nature of the illness and suggesting that muscle abnormalities persist after the acute infection…there is good evidence that Coxsackie B virus is present in the affected muscle in some cases" (*PO Behan, WMH Behan. CRC Crit Rev Neurobiol 1988:4:2:157-178).

1988

*"The main features (of ME) are: prolonged fatigue following muscular exercise or mental strain, an extended relapsing course; an association with neurological, cardiac, and other characteristic enteroviral complications. Coxsackie B neutralisation tests show high titres in 41% of cases compared with 4% of normal adults…These (chronic enteroviral syndromes) affect a young, economically important age group and merit a major investment in research" (*EG Dowsett. Journal of Hospital Infection 1988:11:103-115).

1989

"Ten patients with post-viral fatigue syndrome and abnormal serological, viral, immunological and histological studies were examined by single fibre electromyographic technique….The findings confirm the organic nature of the disease. A muscle membrane disorder…is the likely mechanism for the fatigue and the single-fibre EMG abnormalities. This muscle membrane defect may be due to the effects of a persistent viral infection…There seems to be evidence of a persistent viral infection and/or a viral-induced disorder of the immune system…The infected cells may not be killed but become unable to carry out differentiated or specialised function" (Goran A Jamal, Stig Hansen. Euro Neurol 1989:29:273-276).

1990

*"Skeletal samples were obtained by needle biopsy from patients diagnosed clinically as having CFS (and) most patients fulfilled the criteria of the Centres for Disease Control for the diagnosis of CFS (Holmes et al 1988)…**These data are the first demonstration of persistence of defective virus in clinical samples from patients with CFS**…We are currently investigating the effects of persistence of enteroviral RNA on cellular gene expression leading to muscle dysfunction" (*L Cunningham, RJM Lane, LC Archard et al. Journal of General Virology 1990:71:6:1399-1402).

1990

*"Myalgic encephalomyelitis is a common disability but frequently misinterpreted…**This illness is distinguished from a variety of other post-viral states by a unique clinical and epidemiological pattern characteristic of enteroviral infection**…33% had titres indicative and 17% suggestive of recent CBV infection…Subsequently…31% had evidence of recent active enteroviral infection…**There has been a failure to recognise the unique epidemiological pattern of ME**…Coxsackie viruses are characteristically myotropic and enteroviral genomic sequences have been detected in muscle biopsies from patients with ME. **Exercise related abnormalities of function have been demonstrated by nuclear magnetic resonance and single-fibre electromyography including a failure to coordinate oxidative metabolism with anaerobic glycolysis causing abnormal early intracellular acidosis, consistent with the early fatiguability and the slow recovery from exercise in ME**. Coxsackie viruses can initiate non-cytolytic persistent infection in human cells. Animal models demonstrate similar enteroviral persistence in neurological disease… and **the deleterious effect of forced exercise on persistently infected***

muscles. These studies elucidate the exercise-related morbidity and the chronic relapsing nature of ME" (EG Dowsett, AM Ramsay et al. Postgraduate Medical Journal 1990:66:526-530).

1991

A paper reporting the discovery of a retrovirus associated with (ME)CFS (Retroviral sequences related to human T-lymphotropic virus type II in patients with chronic fatigue immune dysfunction syndrome. Elaine DeFreitas, Paul R Cheney, David S Bell et al. Proc Natl Acad Sci USA 1991:88:2922-2926) is addressed in detail in the section "The role of Viruses in ME/CFS".

1991

*"Persistent enteroviral infection of muscle may occur in some patients with postviral fatigue syndrome and may have an aetiological role….The features of this disorder suggest that the fatigue is caused by involvement of both muscle and the central nervous system…We used the polymerase chain reaction to search for the presence of enteroviral RNA sequences in a well-characterised group of patients with the postviral fatigue syndrome…53% were positive for enteroviral RNA sequences in muscle…Statistical analysis shows that these results are highly significant…On the basis of this study…**there is persistent enteroviral infection in the muscle of some patients with the postviral fatigue syndrome and this interferes with cell metabolism and is causally related to the fatigue"*** (JW Gow et al. BMJ 1991:302:696-696).

1991

*"The findings described here provide the first evidence that postviral fatigue syndrome may be due to a mitochondrial disorder **precipitated by a virus infection**…Evidence of mitochondrial abnormalities was present in 80% of the cases with the commonest change (seen in 70%) being branching and fusion of cristae, producing 'compartmentalisation'. Mitochondrial pleomorphism, size variation and occasional focal vacuolation were detectable in 64%…Vacuolation of mitochondria was frequent…In some cases there was swelling of the whole mitochondrion with rupture of the outer membranes…prominent secondary lysosomes were common in some of the worst affected cases…The pleomorphism of the mitochondria in the patients' muscle biopsies was in clear contrast to the findings in normal control biopsies…Diffuse or focal atrophy of type II fibres has been reported, and this does indicate muscle damage and not just muscle disuse"* (WMH Behan et al. Acta Neuropathologica 1991:83:61-65).

1991

Considerations in the Design of Studies of Chronic Fatigue Syndrome. Reviews of Infectious Diseases. Volume 13, Supplement 1: S1 – S140. University of Chicago Press. Contributing authors included Anthony L Komaroff, David S Bell, Daniel L Peterson, Sandra Daugherty and Sheila Bastien, whose work has been referred to in other parts of this document.

1991

Postviral Fatigue Syndrome. British Medical Bulletin 1991:47:4: 793-907. Churchill Livingstone.

This major publication, published by Churchill Livingstone for The British Council, includes papers by the Wessely School considered by some to be misrepresentative of ME/CFS (for example: "History of postviral fatigue syndrome" by S Wessely; "Postviral fatigue syndrome and psychiatry" by AS David -- in which David, a co-author of the Oxford criteria, confirmed that "*British investigators have put forward an alternative, less strict, operational definition which is essentially chronic…fatigue in the absence of neurological signs, (with) psychiatric symptoms…as common associated features"* (AS David; BMB 1991:47:4:966-988) and "Psychiatric management of PVFS" by M Sharpe) but also contains the following:

"Molecular viral studies have recently proved to be extremely useful. They have confirmed the likely important role of enteroviral infections, particularly with Coxsackie B virus" (Postviral fatigue syndrome: Current neurobiological perspective. PGE Kennedy. BMB 1991:47:4:809-814)

"Our focus will be on the ability of certain viruses to interfere subtly with the cell's ability to produce specific differentiated products as hormones, neurotransmitters, cytokines and immunoglobulins etc in the absence of their ability to lyse the cell they infect. By this means viruses can replicate in histologically normal appearing cells and tissues…Viruses by this means likely underlie a wide variety of clinical illnesses, currently of unknown aetiology, that affect the endocrine, immune, nervous and other …systems" (JC de la Torre, P Borrow, MBA Oldstone. BMB 1991:47:4:838-851).

"*We conclude that persistent enteroviral infection plays a role in the pathogenesis of PVFS…The strongest evidence implicates Coxsackie viruses…Patients with PVFS were 6.7 times more likely to have enteroviral peristence in their muscles"* (JW Gow and WMH Behan. BMB 1991:47:4:872-885).

"The postviral fatigue syndrome (PVFS), with profound muscle fatigue on exertion and slow recovery from exhaustion seems to be related specifically to enteroviral infection. The form seen with chronic reactivated EBV infection is superficially similar, but without the profound muscle fatigue on exercise" (JF Mowbray, GE Yousef. BMB 1991:47:4:886-894).

1992

"We will report at the First International Research Conference on Chronic Fatigue Syndrome to be held at Albany, New York, 2-4 October 1992, our new findings relating particularly to enteroviral infection…We have isolated RNA from patients and probed this with large enterovirus probes…detailed studies...showed that the material was true virus…Furthermore, this virus was shown to be replicating normally at the level of transcription. Sequence analysis of this isolated material showed that it had 80% homology with Coxsackie B viruses and 76% homology with poliomyelitis virus, demonstrating beyond any doubt that the material was enterovirus" (Press Release for the Albany Conference, Professor Peter O Behan, University of Glasgow, October 1992).

1993

"Samples from 25.9% of the PFS (postviral fatigue syndrome) *were positive for the presence of enteroviral RNA, compared with only 1.3% of the controls…We propose that in PFS patients, a mutation affecting control of viral RNA synthesis occurs during the initial phase of active virus infection and allows persistence of replication defective virus which no longer attracts a cellular immune response"* (NE Bowles, RJM Lane, L Cunningham and LC Archard. Journal of Medicine 1993:24:2&3:145-180).

1993

"These data support the view that while there may commonly be asyptomatic enterovirus infections of peripheral blood, it is the presence of persistent virus in muscle which is abnormal and this is associated with postviral fatigue syndrome*…Evidence derived from epidemiological, serological, immunological, virological, molecular hybridisation and animal experiments suggests that persistent enteroviral infection may be involved in… PFS"* (PO Behan et al. CFS: CIBA Foundation Symposium 173, 1993:146-159).

1994

"Individuals with CFS have characteristic clinical and laboratory findings including…evidence of viral reactivation*…The object of this study was to evaluate the status of key parameters of the 2-5A synthetase/RNase L antiviral pathway in individuals with CFS who participated in a placebo-controlled, double-blind, multi-centre trial…The present work confirms the finding of elevated bioactive 2-5A and RNase L activity in CFS…RNase L, a 2-5A-dependent enzyme, is the terminal effector of an enzymatic pathway that is stimulated by either virus infection or*

exposure to exogenous lymphokines. Almost two-thirds of the subjects…displayed baseline RNase L activity that was elevated above the control mean" (Robert J Suhadolnik, Daniel L Peterson, Paul Cheney et al. In Vivo 1994:8:599-604).

A note on the significance of this paper

Viral infections of cells results in the production and secretion of cytokines, including the interferons. Interferons control the way that cells respond to a virus by means of a group of inter-related enzymes that comprise an anti-viral pathway. This pathway is known as the 2',-5'-oligoadenylate synthetase/RNase L pathway.

RNase L (ribonuclease latent) is the key enzyme in the antiviral pathway and is designed to degrade viral RNA. It has to be "turned on" by a small molecule, 2-5 A. Binding of 2-5A to RNase L changes the enzyme from its latent (inactive) state to its active state. When active, RNase L inhibits viral protein synthesis and thereby prevents viral replication.

Several critical parts of the anti-viral pathway are not functioning correctly in ME/CFS.

The level of RNase L enzyme activity has been demonstrated to be upregulated (ie. increased) by as much as 1,500 times above normal levels, and researchers at Temple University School of Medicine, Philadelphia, have shown that not only is the activity of the RNase L enzyme significantly higher in patients with (ME)CFS than in controls, but also that there is a significant increase in the level of 2-5A (the molecule that converts RNase L from its latent to its active state) and in the level of 2-5A synthetase (the enzyme that synthesises the 2-5A activator molecule).

The most striking finding in patients with (ME)CFS is, however, that they have a unique form of the RNase L enzyme. The size of the RNase L protein is normally 80 kDa (kiloDaltons), but in many people with (ME)CFS, this 80 kDa enzyme is either scarce or missing altogether. Instead, a unique low molecular weight (LMW) form of RNase L is observed (30 kDa). Besides its smaller size, the LMW RNase L seen in (ME)CFS patients has other biochemical differences from the 80 kDa RNase L. The LMW RNase L binds its activator more tightly and is more potent than the 80 kDa form of RNase L.

Studies have revealed several connections between the RNase L pathway and the clinical status of (ME)CFS patients, demonstrating that the increased activity of the RNase L pathway is an indication of a lower state of health and that all three measurements of the pathway are abnormal in (ME)CFS.

Studies carried out in various countries apart from the US (including Australia, Belgium, France and Germany) have all confirmed the presence of the LMW RNase L in (ME)CFS; moreover, two different methods using different probes to detect RNase L accurately identified (ME)CFS patients.

Importantly, the RNase L ratio also distinguished individuals with (ME)CFS from those with fibromyalgia or depression.

In addition, studies have shown that the presence of LMW RNase L is independent of the duration of (ME)CFS symptoms: the LMW RNase L was detected in individuals who had (ME)CFS symptoms for as long as 19 years.

The presence of the LMW RNase L identifies a group of people with (ME)CFS who have an abnormally elevated anti-viral response, and the anti-viral RNase L protein level and enzyme activity are potentially powerful diagnostic tools for (ME)CFS (with grateful acknowledgement to Nancy Reichenbach, associate scientist in the Department of Biochemistry at Temple University School of Medicine, and to the CFIDS Association of America: http://www.cfids.org/archives/2000rr/2000-rr1-article01.asp).

Although these important abnormalities were known about in 1994, and despite the evidence of the reliability and reproducibility of RNase L testing that was presented in 1999 at the Second World Congress on (ME)CFS in Brussels, in the UK there has been continued opposition to such testing, not only by the Wessely School (who consistently advise that only limited investigations should be carried out), but also by the ME Association.

For example, the Medical Director of the ME Association, Dr Charles Shepherd, apparently intended to inform readers of the ME Association's Newsletter (Perspectives) that his view of the international work on RNase L was that it *"may involve what I and many of my colleagues regard as over-investigation for highly speculative abnormalities in antiviral pathway activity"*, which seemed to echo Professor Anthony Pinching's view that *"over-investigation can (cause patients) to seek abnormal test results to validate their illness"* (Prescribers' Journal 2000: 40:2:99-106). The Spring 2001 Issue of the ME Association's Medical and Welfare Bulletin stated (on page 9) about RNase L testing: "*Having discussed the possible value of this type of blood test with members of the MEA's Scientific and Medical Advisory Panel, there is general agreement that insufficient evidence exists to recommend that this test should be carried out for either diagnostic or management purposes"* (members of the SMAP included Professor Peter Behan, Professor Leslie Findley, Dr John Gow, Professor Anthony Pinching and Dr Shepherd himself).

The ME Association did, however, co-fund with The Linbury Trust studies examining RNase L activity: blood from patients attending the Fatigue Service at St Bartholomew's Hospital and from Romford, Essex, was sent to Dr John Gow, who was working with Professors Peter and Wilhelmina Behan and Dr Abhijit Chaudhuri, all then at the University of Glasgow. Gow et al's work on a total of 22 patients with CFS was published in Clinical Infectious Diseases (2001:33:12:2080-2081), the conclusion being that *"patients with CFS showed no significant activation"* of either part of the RNase L pathway, and that *"assay of antiviral pathway activation is unlikely to form a rational basis for a diagnostic test for CFS".*

Professors Suhadolnik and De Meirleir robustly showed that Gow et al's study was fundamentally flawed. Pointing out that *"Over the years, our teams have repeatedly observed an activation at the enzymatic level of the antiviral pathway in subsets of CFS patients",* they noted that Gow et al had (1) misunderstood the established knowledge of the IFN pathway, (2) did not confirm their observations of genetic expression <u>at the transcriptional level</u> (which would have clarified their results), (3) used the terms "genetic expression" and "activity" interchangeably, when they are not necessarily synonymous (particularly when the research involves enzymes). They also noted that confusion in the mind of Gow et al about these issues led them to misquote their articles: "*On the basis of their limited observations, Gow et al challenge our observations and further deny any rational basis to our proposal regarding the use of 37-kDa RNase L detection as a biological marker for CFS. In our study, which they clearly misquoted, we did not measure the enzymatic activity of the fragment and, hence, the 2-5A pathway activation as Gow and colleagues claimed. Instead, we limited our study to the quantitative detection of the 37-kDa truncated enzyme…We observed a significant increase in the 37-kDa RNase L level in patients with CFS compared with that observed in healthy control subjects, patients with fibromyalgia, and patients with depression….Consequently, this does not support the claim that the presence of the 37-kDa RNase L in CFS could only be imparted to non-specific increases in the antiviral pathway activation…Our data demonstrate that there is a more comprehensive downstream cellular role for the signal transduction by IFN than what Gow and colleagues pretend to present to the readers of Clinical Infectious Diseases"* (Clin Inf Dis 2002:34:1420-1421).

The ME Association and its medical advisors, however, remained convinced that Gow et al were correct: "*A very important conclusion from this study is that costly investigations such as the RNase L test, which assess the amount of antiviral activity in ME/CFS, are unlikely to provide the basis for a diagnostic test. Such tests are therefore of very questionable value in the assessment of people with ME/CFS"* (MEA Medical and Welfare Bulletin, Spring 2002, Issue No 6, page 10).

At the AACFS International Research Conference in 2003 held in Washington, Wilhelmina Behan, as co-author of the Gow et al study, was publicly challenged by Professor Suhadolnik to defend it, but was unable to do so.

Notwithstanding, on the basis of the Gow / Behan results, the ME Association's Medical Advisor remains of the view that *"the presence of …abnormalities in antiviral pathways has been assessed in research studies funded by the ME Association"* and that the results of these tests are not *"of proven value"* (ME/CFS/PVFS: An exploration of the key clinical issues. Dr Charles Shepherd and Dr Abhijit Chaudhuri, for The ME Association, 2007).

In contrast to such UK views about the significance of RNase L, in 2000 Professor Anthony Komaroff from Harvard had written about Professor De Meirleir's work on RNase L in an Editorial in the American Journal of Medicine: *"What is this research telling us? It is another piece of evidence that the immune system is affected in chronic fatigue syndrome and it reproduces and extends the work of another investigator (Professor Suhadolnik from the US), lending credibility to the result"* (Am J Med 2000:108:169-171).

It is worth noting that elevated levels of RNase L are associated with reduced maximal oxygen consumption (VO_2 max) and exercise duration in ME/CFS patients; Snell et al found that both abnormal RNase L activity and low oxygen consumption were observed in most (ME)CFS patients, findings that demonstrate that patients' extremely low tolerance for physical activity is likely to be linked to abnormal oxidative metabolism, perhaps resulting from defective interferon responses (Comparison of maximal oxygen consumption and RNase L enzyme in patients with CFS. C Snell et al. AACFS Fifth International Research and Clinical Conference, Seattle, January 2001; #026).

It is also worth noting that the 37 kDa LMW RNase L fragment found in ME/CFS patients is produced by cleavage of calpain (an apoptotic enzyme), and the whole process affects the calcium and potassium ion channels, a channelopathy that will lead to low body potassium (a known finding in ME-CFS patients -- Burnett et al found that total body potassium (TBK) was lower in patients with (ME)CFS and suggest that abnormal potassium handling by muscle in the context of low overall body potassium may contribute to fatigue in (ME)CFS (Medical Journal of Australia, 1996:164:6:384).

It is also important to note that patients who express the low molecular weight RNase L may have problems with enzymatic detoxification pathways, particularly in the liver. This is significant because of the resultant adverse effect on thyroid function.

It has long been noted by practitioners that ME/CFS patients are often clinically hypothyroid even though biochemically euthyroid. Evidence suggests that such patients may not really be euthyroid, especially at the tissue level. (Chopra IJ. J Clin Endocrinol Metab 1997:82(2):329-334), so particular attention needs to be paid to investigating the bioavailablity of T3 because in ME/CFS, T3 levels are often low (or at the low end of the normal range). Consequently, selenium levels need to be investigated in patients with ME/CFS who have reduced T3 levels: this is because selenium (as selenocysteine) is an integral component of two important enzymes, glutathione peroxidase and iodothryonine deiodinase; it is expressed in the liver and it regulates the conversion of thyroxine (T4) to the active and more potent T3. Individuals who have a deficiency of 5' deiodinase cannot produce T3 from T4, thus it is necessary to establish baseline levels of selenium in ME/CFS patients whose T3 levels are low.

In the UK, the NICE Guideline does not recommend such testing.

In relation to RNase L, a recent literature review of the immunological similarities between cancer and (ME)CFS pointed out:

"Cancer and CFS are both characterised by fatigue and severe disability (and) certain aspects of immune dysfunctions appear to be present in both illnesses…A literature review of overlapping immune dysfunctions in CFS and cancer is provided. ***Abnormalities in ribonuclease (RNase L)*** *and hyperactivation of nuclear factor kappa-beta (NF-kappaβ) are present in CFS and in prostate cancer. Malfunctioning of natural killer (NK) cells has long been recognised as an important factor in the development and recurrence of cancer, and has been documented repeatedly in CFS patients.* ***The dysregulation of the RNase L pathway****, hyperactive NF-kappaβ leading to disturbed apoptotic mechanisms and oxidative stress or excessive nitric oxide, and low NK activity may play a role in the two diseases (and)… are*

present in both diseases. These anomalies may be part of the physiopathology of some of the common complaints, such as fatigue" (Meeus M et al. Anticancer Res 2009:29(11):4717-4726).

It seems that, even if not a specific biomarker for ME/CFS, the significance of the abnormal RNase L anti-viral pathway in ME/CFS patients cannot be sufficiently emphasised, but through the undoubted influence of the Wessely School, ME/CFS sufferers in the UK are not permitted to have their anti-viral pathway status investigated.

1994

Chronic Fatigue Syndrome: Current Concepts. Clinical Infectious Diseases 1994: Volume 18: Supplement 1: S1 – S167. Ed. Paul H Levine. University of Chicago Press. Contributing authors include: Paul H Levine, Alexis Shelokov, Anthony L Komaroff, David S Bell, Paul R Cheney, Leonard H Calabrese, Leonard A Jason, Seymour Grufferman, Hirohiko Kuratsune, Charles Bombadier, Nancy G Klimas, Mary Ann Fletcher, Roberto Patarca-Montero, Benjamin H Natelson, Robert J Suhadolnik, Daniel L Peterson, Dharam V Ablashi, Fred Friedberg, Jay A Levy, Peter O Behan, Wilhelmina MH Behan and Mark O Loveless.

In his Summary of the Viral Studies of CFS, Dr Dharam V Ablashi concluded: ***"The presentations and discussions at this meeting strongly supported the hypothesis that CFS may be triggered by more than one viral agent…Komaroff suggests that, once reactivated, these viruses contribute directly to the morbidity of CFS by damaging certain tissues and indirectly by eliciting an on-going immune response"*** (Clin Inf Dis 1994:18 (Suppl 1):S130-133). It is recommended that the entire 167-page Journal be read to show how ill-founded is the Wessely School's "CBT model" of ME/CFS.

In their Closing Remarks, Professors Komaroff and Klimas said: "***Few studies by psychiatrists are presented in this supplement. Many investigators who have argued that CFS is primarily a psychiatric disorder chose not to present their work***" (Clin Inf Dis 1994:18:(Suppl 1):S166-167).

1995

*"These results suggest there is persistence of enterovirus infection in some CFS patients and indicate the presence of distinct novel enterovirus sequences…****Several studies have shown that a significant proportion of patients complaining of CFS have markers for enterovirus infection****….From the data presented here…the CFS sequences may indicate the presence of novel enteroviruses…****It is worth noting that the enteroviral sequences obtained from patients <u>without</u> CFS were dissimilar to the sequences obtained from the CFS patients…This may provide corroborating evidence for the presence of a novel type of enterovirus associated with CFS****"* (DN Galbraith, C Nairn and GB Clements. Journal of General Virology 1995:76:1701-1707).

1995

"In the CFS study group, 42% of patients were positive for enteroviral sequences by PCR, compared to only 9% of the comparison group…Enteroviral PCR does, however, if positive, provide evidence for circulating viral sequences, and has been used to show that ***enteroviral specific sequences are present in a significantly greater proportion of CFS patients than other comparison groups****"* (C Nairn et al. Journal of Medical Virology 1995:46:310-313).

1997

"To prove formally that <u>persistence</u> rather than re-infection is occurring, it is necessary to identify a unique feature retained by serial viral isolates from one individual. ***We present here for the first time evidence for enteroviral persistence (in humans with CFS****)…" (*DN Galbraith et al. Journal of General Virology 1997:78:307-312).

1998

"Recent developments in molecular biology…have revealed a hitherto unrecognised association between enteroviruses and some of the most disabling, chronic and disheartening neurological, cardiac and endocrine diseases… ***Persistent infection (by enteroviruses) is associated with ME/CFS****…The difficulty of making a differential diagnosis between ME/CFS and post-polio sequelae cannot be over-emphasised…*(EG Doswett. Commissioned for the BASEM meeting at the RCGP, 26th April 1998:1-10).

2000

An important paper by Ablashi and Peterson et al suggested that in both multiple sclerosis (MS) and (ME)CFS, HHV-6 reactivation plays a role in the pathogenesis.

"***Two disorders of significant importance, MS and CFS, appear to be associated with HHV-6 infection…the data presented here show that both MS and CFS patients tend to carry a higher rate of HHV-6 infection or reactivation compared to normal controls. This immunological and virological data supports a role of HHV-6 in the symptomatology of these diseases****…Based on biological, immunological and molecular analysis, the data show that HHV-6 isolates from 70% of CFS patients were Variant A…Interestingly, the majority of HHV-6 isolates from MS patients were Variant B…These data demonstrate that the CFS patients exhibited HHV-6 specific immune responses…****Seventy percent of the HHV-6 isolates from CFS patients were Variant A, similar to those reported in AIDS…It has already been shown that active HHV-6 infection in HIV-infected patients enhanced the AIDS disease process. We suspect that the same scenario is occurring in the pathogenesis of MS and CFS****…The immunological data presented here clearly shows a significantly high frequency of HHV-6 reactivation in CFS and MS patients. We postulate that active HHV-6 infection is a major contributory factor in the aetiologies of MS and CFS" (*DV Ablashi, DL Peterson et al. Journal of Clinical Virology 2000:16:179-191).

(HHV-6 is one of eight known members of the human herpesvirus family. It has two variants [A and B]; the A strain is much more pathogenic and infects the immune and central nervous systems. Reactivation in adults has been associated with glandular fever, autoimmune disorders and diseases of the nervous system. Active HHV-6 infections are not found in healthy people without disease associations and reactivation can result in suppression of bone marrow function and inflammation, and can cause damage in tissues such as brain, liver or lungs. HHV-6 has been specifically linked to MS, AIDS and (ME)CFS [Co-Cure MED: 2nd March 2002]. HHV-6 used to be called human B-lymphotropic virus (HBLV); it was discovered in 1986 from the blood of patients with AIDS. HHV-6 also correlates with 37kDa – the low molecular weight form of RNase L that is known to exist as part of a dysregulated antiviral pathway in ME/CFS patients).

2001

"Over the last decade a wide variety of infectious agents has been associated with CFS by researchers from all over the world. Many of these agents are neurotrophic and have been linked to other diseases involving the central nervous system (CNS)*…Because patients with CFS manifest a wide range of symptoms involving the CNS as shown by abnormalities on brain MRIs, SPECT scans of the brain and results of tilt-table testing, we sought to determine the prevalence of HHV-6, HHV-8, EBV, CMV, Mycoplasma species, Chlamydia species and Coxsackie virus in the spinal fluid of a group of patients with CFS. Although we intended to search mainly for evidence of actively replicating HHV-6, a virus that has been associated by several researchers with this disorder,* ***we found evidence of HHV-8, Chlamydia species, CMV and Coxsackie virus in (50% of patient) samples…It was also surprising to obtain such a relatively high yield of infectious agents on cell free specimens of spinal fluid that had not been centrifuged****" (*Susan Levine. JCFS 2002:9:1/2:41-51).

(HHV-8 is associated with Kaposi's sarcoma which is found in HIV AIDS and with some B-cell lymphomas).

2003

Nicolson et al showed that multiple co-infections (Mycoplasma, Chlamydia, HHV-6) in blood of chronic fatigue syndrome patients are associated with signs and symptoms: "***Differences in bacterial and/or viral infections in (ME)CFS patients compared to controls were significant…The results indicate that a large subset of (ME)CFS patients show evidence of bacterial and/or viral infection(s)****, and these infections may contribute to the severity of signs and symptoms found in these patients"* (Nicolson GL et al. APMIS 2003:111(5):557-566).

2003

Seeking to detect and characterise enterovirus RNA in skeletal muscle from patients with (ME)CFS and to compare efficiency of muscle metabolism in enterovirus positive and negative (ME)CFS patients, Lane et al obtained quadriceps biopsy samples from 48 patients with (ME)CFS. Muscle biopsy samples from 20.8% of patients were positive, while 100% of the controls were negative for enterovirus sequences. Lane et al concluded: "*There is an association between abnormal lactate response to exercise, reflecting impaired muscle energy metabolism, and the presence of enterovirus sequences in muscle in a proportion of (ME)CFS patients"* (RJM Lane, LC Archard et al. JNNP 2003:74:1382-1386).

2005

In their presentation to the US Assembly Committee, Drs Dharam Ablashi and Kristin Loomis said:

*"**Reasons to suspect viruses as a cause of CFS and MS**: **In CFS, symptoms wax and wane; antiviral pathways are activated; symptoms are similar to many viral conditions**; geographic outbreaks have been reported; **gene expression profiling found genetic variants that reduce antiviral defences.** In MS, antiviral pathways are activated; geographic outbreaks have been reported; all demyelinating disorders with known aetiology have been caused by viruses; symptoms wax and wane and worsen with viral infections.*

"Evidence of central nervous system abnormalities in (ME)CFS are similar to those in MS: reduced grey matter volume in bilateral prefrontal cortex; abnormal uptake of acetyl-L carnitine in the prefrontal cortex; enlarged ventricle volumes; increased small punctate lesions on MRI in MS and in a subset of (ME)CFS; fatigue is present in more than 85% of people with MS and in 100% of people with (ME)CFS; reduced information processing speed; memory and cognitive problems".

Ablashi and Loomis pointed out that **an analysis of studies of HHV-6 in (ME)CFS differentiated between active and latent virus, with 83% being positive** (Assessment and Implications of Viruses in Debilitating Fatigue in CFS and MS Patients. Dharam V Ablashi et al. HHV-6 Foundation, Santa Barbara, USA. Submission to Assembly Committee/Ways & Means, Exhibit B1-20, submitted by Annette Whittemore 1st June 2005).

2005

In a review of the role of enteroviruses in (ME)CFS, Chia noted that initial reports of chronic enteroviral infections causing debilitating symptoms in (ME)CFS patients were met with scepticism and largely forgotten, but observations from *in vitro* experiments and from animal models clearly established a state of chronic persistence through the formation of double stranded RNA, similar to findings reported in muscle biopsies of patients with (ME)CFS. Recent evidence not only confirmed the earlier studies, but also clarified the pathogenic role of viral RNA (JKS Chia. Journal of Clinical Pathology 2005:58:1126-1132).

2006

"We now recognise that the immune system plays a crucial role in the pathogenesis of (ME)CFS…A disruption of the HPA axis has been implicated in the pathogenesis of (ME)CFS…A link between the immune system and the HPA axis has long been established…it is likely that HPA axis dysfunction is not the cause of (ME)CFS, but that it is secondary to the primary pathogenesis. However, once invoked, HPA axis dysfunction may contribute towards the perpetuation of the illness…Stress is known to have a significant modulating effect on the pathogenesis of viral infection (and) the principal means by which this influence occurs is likely to be via the HPA axis…Early beliefs that (ME)CFS may be triggered or caused by a single virus have been shown to be unsubstantiated (and) it is likely that different viruses affect different individuals differently, dependent upon the …immune competence of the individual… ***Infections are known to trigger and perpetuate the disease in many cases. Therefore, one valuable approach that has not been widely adopted in the management of (ME)CFS patients is to exhaustively investigate such patients in the hope of identifying evidence for a specific persistent infection*** (but in the UK, NICE specifically does not permit such investigations)…. ***Enteroviruses have been reported to trigger approximately 20% of cases if (ME)CFS…Antibodies to Coxsackie B virus are frequently detected in (ME)CFS patients, and enterovirus protein and RNA occur in the muscle and blood of (ME)CFS patients and their presence has been associated with altered metabolism in the muscle upon exercise in the context of (ME)CFS****".*

Kerr et al then go on to provide evidence of other triggers of (ME)CFS which include Parvovirus; *C. pneumoniae*; *C. burnetti;* toxin exposure and vaccination including MMR, pneumovax, influenza, hepatitis B, tetanus, typhoid and poliovirus (LD Devanur, JR Kerr. Journal of Clinical Virology 2006: 37(3):139-150).

2006

Having carried out a prospective cohort study of post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens, the authors concluded: *"****The syndrome was predicted largely by the severity of the acute illness rather than by demographic, psychological or microbiological factors****…****Importantly, premorbid and intercurrent psychiatric disorder did not show predictive power for post-infective fatigue at any time point…We propose that …neurobiological mechanisms triggered during the severe, acute illness…underpin the persistent symptoms domains of post-infective fatigue syndrome****"* (Ian Hickie et al. BMJ 2006: 333:575).

2006

*"CFS is a poorly-defined medical condition…which, besides severe chronic fatigue as the hallmark symptom, involves inflammatory and immune activation…****The type I interferon antiviral pathway has been repeatedly shown to be activated in peripheral blood mononuclear cells of the most severely afflicted patients…Recently, the levels of this abnormal protein have been significantly correlated to the extent of inflammatory symptoms displayed by (ME)CFS patients.*** *We report here that active double-stranded RNA-dependent kinase (PKR) is expressed and activated in parallel to the presence of the 37 kDa RNase L and to an increase in nitric oxide production by immune cells…****These results suggest that chronic inflammation due to excess nitric oxide production plays a role in (ME)CFS and that the normal resolution of the inflammatory process by NFK-β activation and apoptotic induction is impaired"*** (Marc Fremont, Kenny De Meirleir et al. JCFS 2006:13:4:17-28).

2006

"(ME)CFS is associated with objective underlying biological abnormalities, particularly involving the nervous and immune system. ***Most studies have found that active infection with HHV-6 – a neurotropic, gliotropic and immunotropic virus – is present more often in patients with (ME)CFS than in healthy control subjects****…Moreover, HHV-6 has been associated with many of the neurological and immunological findings in patients with (ME)CFS"* Anthony L Komaroff. Journal of Clinical Virology 2006:37:S1:S39-S46.

2007

*"**Research studies have identified various features relevant to the pathogenesis of CFS/ME such as viral infection, immune abnormalities and immune activation, exposure to toxins, chemicals and pesticides, stress, hypotension…and neuroendocrine dysfunction….Various viruses have been shown to play a triggering or perpetuating role, or both, in this complex disease….The role of enterovirus infection as a trigger and perpetuating factor in CFS/ME has been recognised for decades**…The importance of gastrointestinal symptoms in CFS/ME and the known ability of enteroviruses to cause gastrointestinal infections led John and Andrew Chia to study the role of enterovirus infection in the stomach of CFS/ME patients…They describe a systematic study of enterovirus infection in the stomach of 165 CFS/ME patients, demonstrating a detection rate of enterovirus VP1 protein in 82% of patients…the possibility of an EV outbreak…seems unlikely, as these patients developed their diseases at different times over a 20 year period"* (Jonathan R Kerr. Editorial. J Clin Pathol 14th September 2007. Epub ahead of print).

2007

"*Since most (ME)CFS patients have persistent or intermittent gastrointestinal (GI) symptoms, the presence of viral capsid protein 1 (VP1), enterovirus RNA and culturable virus in the stomach biopsy specimens of patients with (ME)CFS was evaluated…Our recent analysis of 200 patients suggests that… enteroviruses may be the causative agents in more than half of the patients…At the time of oesophagogastroduodenoscopy, the majority of patients had mild, focal inflammation in the antrum…95% of biopsy specimens had microscopic evidence of mild chronic inflammation…82% of biopsy specimens stained positive for VP1 within parietal cells, whereas 20% of the controls stained positive…An estimated 80-90% of our 1,400 (ME)CFS patients have recurring gastrointestinal symptoms of varying severity, and epigastric and/or lower quadrant tenderness by examination…Finding enterovirus protein in 82% of stomach biopsy samples seems to correlate with the high percentage of (ME)CFS patients with GI complaints…Interestingly, the intensity of VP1 staining of the stomach biopsy correlated inversely with functional capacity…A significant subset of (ME)CFS patients may have a chronic, disseminated, non-cytolytic form of enteroviral infection which can lead to diffuse symptomatology without true organ damage" (*Chia JK, Chia AY. J Clin Pathol 13th September 2007 Epub ahead of print).

2009

As mentioned elsewhere, researchers from the Enterovirus Research Laboratory, Department of Pathology and Microbiology, University of Nebraska Medical Centre wrote a specially-commissioned explanatory article for the UK charity Invest in ME, in which they stated that human enteroviruses were not generally thought to persist in the host after an acute infection, but they had discovered that Coxsackie B viruses can naturally delete sequence from the 5′ end of the RNA genome, and that this results in long-term viral persistence, and that *"This previously unknown and unsuspected aspect of enterovirus replication provides an explanation for previous reports of enteroviral RNA detected in diseased tissue in the apparent absence of infectious virus particles" (*S Tracy and NM Chapman. Journal of IiME 2009:3:1).
(http://www.investinme.org/Documents/Journals/Journal%20of%20IiME%20Vol%203%20Issue%201.pdf).

In her lecture in November 2009 at the University of Miami, Professor Nancy Klimas said about viruses and ME/CFS that much of the research at Miami and internationally found that the viruses studied all have several things in common: they infect cells of the immune system and the neurological system; they are capable of causing latent infections and they can reactivate under certain conditions.

She also said that their early work at Miami in the late 1980s (published in the Journal of Clinical Microbiology in 1989) showed that ME/CFS patients had immune activation and poor anti-viral cell function. She then went on to discuss the importance of the findings of the retrovirus XMRV (evidence of which was published in Science on 8th October 2009), saying that it was *"very impressive work"*. She continued: "*This Science paper was amazing for a number of reasons. First, this team had put together such strong science that they could go for a Science paper. Science is like the Mecca of publication. If you get your stuff in Science,*

that's the best place you could possibly (get it published). And they don't take just anything and they sure, sure, sure don't take anything unless it's extremely well done, validated and tested out. So they took this paper – they not only took it, they put it in Science Express. They thought it was so important, they published on a very fast track…The way (the researchers at the Whittemore Peterson Institute) looked is very sophisticated…They then tried to find (the virus) in all these other ways…they looked from a whole different angle. Still found it. Backed up and looked from another angle. Still found it…they had five different kinds of ways they looked for this virus. And they were able to find the virus. That's why Science was so impressed…It is a virus that can infect tissues that aren't white blood cells… ***We've always thought something like that has to go on in (ME)CFS because you all have some neuro-inflammation. Your brain has a low grade level of inflammation. And you have some inflammation in the tissues that make hormones, particularly in the hypothalamic-pituitary-axis. And this is a virus that infects that type of tissue****…It's pretty impressive that out of 101 (ME)CFS cases defined by clinical case definition or a research case definition that they found 99 with the virus…And, oh, by the way, we have a biomarker. Not a small deal. A biomarker – the virus itself. No better biomarker than something that's clearly, tightly associated with an illness…So the conclusion, it really is a big thing. It's a big thing…That work we were already doing plays right into this. All the genomics work and all the immunology work. This is all critical to the better understanding of this illness and how this virus plays into it"* (with grateful acknowledgement to PANDORA and http://aboutmecfs.org/Rsrch/XMRVKlimas.aspx and http://aboutmecfs.org/Rsrch/XMRVKlimasII.aspx).

The Whittemore-Peterson Institute's study that found the new human retrovirus XMRV was listed as one of the top 100 scientific discoveries in 2009 in Discovery magazine's January 2010 issue (Co-Cure NOT: 30th December 2009).

There can be no dismissing the evidence of viral involvement in ME/CFS, much of which pre-dated the PACE Trial.

The Trial Investigators, the MRC Data Monitoring and Ethics Committee, the Trial Steering Committee and the Trial Management Group surely have a duty to provide a convincing explanation for their decision not to inform Trial participants of this fundamental evidence as, without it, participants may not have been in a position to provide fully informed consent before agreeing to enter the Trial.