Cytokines in Parvovirus B19 Infection As an Aid to Understanding Chronic Fatigue Syndrome

Jonathan R. Kerr, MD and David A.J. Tyrrell, MD†*

Address
*Department of Microbiology, Royal Brompton Hospital, National Heart & Lung Institute, Imperial College London, Sydney Street, London SW3 6NP, UK.
E-mail: j.kerr@imperial.ac.uk
†CFS Research Foundation, Salisbury, Wiltshire.

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Human parvovirus B19 infection has been associated with various clinical manifestations of a rheumatic nature such as arthritis, fatigue, and chronic fatigue syndrome (CFS), which can persist for years after the acute phase. The authors have demonstrated recently that acute B19 infection is accompanied by raised circulating levels of IL-1β, IL-6, TNF-α, and IFN-γ and that raised circulating levels of TNF-α and IFN-γ persist and are accompanied by MCP-1 in those patients who develop CFS. A resolution of clinical symptoms and cytokine dysregulation after intravenous immunoglobulin (IVIG) therapy, which is the only specific treatment for parvovirus B19 infection, also has been reported. Although CFS may be caused by various microbial and other triggers, that triggered by B19 virus is clinically indistinguishable from idiopathic CFS and exhibits similar cytokine abnormalities and may represent an accessible model for the study of CFS.

Introduction

It has been reported recently that chronic fatigue and joint pain may follow acute symptomatic parvovirus B19 infection, which, in certain cases, fulfills diagnostic criteria for chronic fatigue syndrome (CFS) and that CFS follows symptomatic B19 infection with an incidence of 13% [1••]. In addition, patients with B19-associated CFS were shown to have raised circulating levels of tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) in comparison with normal controls [2••]. In this review, the significance of these findings in our understanding and further investigation of the pathogenesis of CFS and related conditions are discussed.

Chronic Fatigue Syndrome

Fatigue is a common perceived symptom for the general population, with a prevalence of up to 50% [3]. This fatigue typically is short-lived and self-limited and may be associated with current circumstances. However, CFS is an illness characterized by disabling fatigue for at least 6 months, which is accompanied by various rheumatologic, infectious, and neuropsychiatric symptoms [4]. CFS is more common in women than in men. Chronic fatigue and related symptoms often may occur concurrently with several other functional conditions such as fibromyalgia (FM), irritable bowel syndrome, temporomandibular joint disorder, and multiple chemical sensitivity [5,6]. FM is defined by the presence of symptoms of widespread musculoskeletal pain, stiffness, and pressure hyperalgesia at particular soft tissue sites for at least 3 months [7]. However, these criteria of the American College of Rheumatology (ACR) focus only on pain, not on other important symptoms that are associated commonly with FM (*eg,* fatigue, cognitive disturbance, sleep abnormalities, and psychologic distress) [8] and are responsible for blurring the divide between FM and CFS. Similar to CFS, FM is more common in women than in men and occurs mostly in patients who are middle aged.

Case Definition for Chronic Fatigue Syndrome

The diagnosis of CFS is symptom-based and performed according to established case definitions; the best known and most widely used is that developed by the Centers for Disease Control (CDC) [4]. This definition requires at least 6 months of persistent fatigue with a consequent and significant reduction in one or more of the following areas of the patient's life: occupational, educational, social, and personal. In addition, four or more of the following symptoms must occur with fatigue in a 6-month period: impaired memory/concentration, sore throat, sore or stiff muscles, tender lymph glands, polyarthralgia, new headaches, nonrefreshing sleep, and postexertional fatigue. Other conditions that must be excluded to enable a diagnosis of CFS include those that may cause fatigue, such as rheumatoid arthritis (RA), systemic lupus erythematosus, psychosis, bipolar

disorder, major depression, eating disorders, and substance abuse within 2 years of the onset of fatigue.

The Approach to the Study of Chronic Fatigue Syndrome

In recent decades, the study of CFS has followed two main tracks: whether there was such a condition and, if so, how to recognize and define it. Various definitions were introduced, but an international consensus was finally reached that it could be recognized by the symptom complex of fatigue, which was disabling and lasting; there often are some associated symptoms, but no clinical or laboratory evidence of recognized disabling medical or psychiatric conditions [4]. These criteria of the CDC are endorsed widely for research purposes. Some groups distinguish postinfectious or postviral CFS; it is common after Epstein-Barr virus (EBV) infections [9]. However, a preceding infection is not documented often, but may be based on a clinical impression only, although it has been shown recently that intensive testing may reveal the infectious nature of many cases of CFS [10], diagnoses that might be missed otherwise.

The second track is to use sensitive tests of the immunologic and neurologic systems. These have been applied, in most cases, to patients who have been recognized clinically. In the 1980s, it was found that various tests showed that the lymphocytes were activated during active disease and corticosteroid control was altered. However, in addition, physiologic studies support the patients' stories that sleep rhythms and some measures of cerebral function were disturbed [11].

Most observers think that the CFS case definition includes a number of different conditions as judged by the details of the history and symptomatology. This is supported by the fact that these are cases that overlap the distinction between FM and CFS. It also is supported by research, which shows that, in some cases, there is laboratory-supported evidence that the illness follows or accompanies infections with EBV and enteroviruses, *Coxiella burnetii* (the agent of Q fever), or hepatitis, although in individual cases, it often is not possible to prove that the infection triggered the syndrome.

Therefore, it is an attractive hypothesis that CFS, particularly the characteristic fatigue, can be the result of some unusual immune response (possibly to an infection). Particularly in the past decade, it has become known that immune responses are mediated and modulated by the release of a wide range of chemical messengers or cytokines. There are receptors for these molecules on cells of the nervous system. The effects may be demonstrated in the laboratory; clinical work shows that when the purified synthetic molecules such as interferons and interleukins are administered to patients or healthy volunteers, there is a general response including malaise and fatigue. Therefore, it is plausible that, in at least some CFS cases, the main symptoms may result from an abnormal release of these molecules. There have been investigations of the details of immune system behavior in CFS and the presence of cytokines in the circulation. Although the results are neither consistent nor reproducible, enough of them are positive to encourage further studies.

Immune Function and Cytokines in Chronic Fatigue Syndrome

Patients with CFS have been shown to have evidence of immune activation comprised by increased numbers of activated T lymphocytes, cytotoxic T lymphocytes, and elevated levels of circulating cytokines [12–14]. Decreases in NK cell cytotoxic and lymphoproliferative activity along with increased autoimmune and allergic manifestations in the CFS support the hypothesis that the immune system in CFS is biased towards a T-helper (Th) 2 profile, with a predominance of humoral immunity [15]. However, the factors that may lead to such a Th2 shift, along with mood and immunoendocrine changes in patients with CFS, are unclear [16].

Accentuated interleukin (IL)-6 production by stimulated peripheral blood mononuclear cell (PBMC) has been demonstrated in CFS [13,14] and Q fever-related CFS (QFS) [17]. IL-6 modulates activity of the hypothalamic-pituitary-adrenal axis, induces disorders of serotonin metabolism, and may mediate the behavior associated with severe depression [18–20]. In laboratory models, type 1 and 2 cytokines and IL-6 can regulate levels of neurotransmitters (*eg*, monoamines and nitric oxide) [21,22]. Three of four studies found that transforming growth factor-β (TGF-β) levels were significantly higher in patients with CFS compared with control subjects [13,14,23,24]. TGF-β is an immunosuppressive cytokine and may be exploited by an infectious agent to facilitate persistence. Several studies have shown that patients with CFS have elevated levels of serum IFN-γ [25] and TNF-α [26,27] compared with control subjects. However, these findings are not invariable [24].

The hypothesis of chronic immune activation in CFS has been supported further by the observation that clinical treatment with recombinant cytokines such as IL-1, IL-2, and IFN-γ gave rise to many of the symptoms of CFS [28–31]. AntiTNF-α drugs have been shown to lead to an improvement in fatigue and mood [32]. In addition, ex vivo treatment of lymphocytes with IL-2 followed by autologous transfusion of these led to clinical improvement accompanied by evidence of enhancement of Th1 bias in nine of 11 subjects [33].

Cytokine abnormalities also have been documented in patients with FM. For example, raised levels of IL-6 and IL-8 have been demonstrated in FM patient sera and PBMC compared with control subjects [34,35]. Patients with FM are reported to have a tendency towards cold extremities

and cold-induced vasospasm; one report documents an increased level of plasma endothelin-1 [36].

Microbial Triggers of Chronic Fatigue Syndrome

Epidemiologic studies have revealed that many CFS patients have a history of an illness consistent with viral infection, which precedes the development of fatigue [11]. In addition, CFS has been shown to follow acute infection with various infectious agents, including EBV, and CFS has even been referred to as chronic glandular fever [9], enteroviruses [37], *Coxiella burnetii* [38], parvovirus B19 [1••], viral meningitis [39], viral hepatitis [40], *Chlamydia pneumoniae*, cytomegalovirus, recurrent varicella-zoster virus infection, human herpes virus-6, hepatitis C, toxic mold exposure, vaccination with measles, mumps, and rubella, pneumovax, and influenza vaccine [10]. After 20 years of research to find a possible microbial cause for CFS, it is increasingly clear that no single infectious agent is responsible [10]. It has been proposed that we should approach CFS in the same way that we approach fever of unknown origin, to etiologically diagnose as many cases as possible and to treat each infection appropriately [10,41].

Human Parvovirus B19

Human parvovirus B19, discovered in 1975 [42], is a very small (23 nm in diameter) single-stranded DNA virus that usually is transmitted by respiratory aerosol droplet spread. In the several years after the discovery of the virus, the only diagnostic tool was electron microscopy, which was time consuming and technically demanding. With the advent of B19-specific monoclonal antibodies, immunoassays were introduced. Subsequent seroprevalence studies documented that the peak incidence occurs in childhood and early adolescence and that adults have a seroprevalence of 60% to 85%. In addition to the peaks of incidence that occur in spring and early summer, virus infections show longer cycles with peak prevalences occurring every 4 to 5 years. B19 virus causes infection throughout the world with the exception of certain isolated tribes. Although infection, and sometimes replication, has been demonstrated in other cells, the primary target cells for B19 virus are erythroblasts, which are found in the bone marrow and are the precursors for mature erythrocytes. The virus receptor on these cells has been shown to be blood group P antigen (globoside) [43], which also occurs on the surface of various other cells [44] and mediates virus attachment.

The B19 infection has been associated with various clinical manifestations. B19 infection causes erythema infectiosum (fifth disease) and is commonly associated with transient aplastic crisis in patients with shortened red cell survival, arthritis, which may be severe, prolonged and is sometimes confused with RA, fetal death after maternal infection, which usually is found to begin during the second trimester, and persistent anemia in immunocompromised patients. Infection is associated less commonly with a range of diverse skin rashes, hematologic manifestations such as cytopenias and aplastic anemia, neurologic manifestations such as meningoencephalitis, hepatobiliary disease, and rheumatic diseases including CFS [45].

The immune system has been implicated in the mediation of a number of clinical manifestations of parvovirus B19 infection including rash and arthralgia [46], fatigue [1••,2••], and glomerulonephritis [47], and autoimmunity has shown an increasingly prominent association with B19 infection [48–51]. The humoral immune response is thought to represent the major defense against B19 virus similar to the way specific antibody protects against infection in vivo and in vitro and to the way human normal immunoglobulin frequently clears virus from peripheral blood and results in clinical improvement in immunosuppressed patients with persistent infection [52,53]. In addition, lymphoproliferative responses have been demonstrated against parvovirus VP1/2 capsid antigens in patients with previous B19 infection [54]. The particular progression of these events in a patient may be mediated by the type of CD4+ T-cell response [55,56], which has been shown for other viruses [57,58].

Because B19-associated disease may be varied, prolonged, and result from direct virus infection and the specific immune response, the authors' approach to the study of parvovirus B19-associated disease has been to follow patients from the acute phase and observe them at a later date to determine those symptoms that originated from the time of acute infection and their duration. In addition, this approach also facilitates laboratory investigations at various times. The contribution to CFS made by subclinical parvovirus infections has not been addressed.

Parvovirus B19-associated Fatigue and Chronic Fatigue Syndrome

Several groups have documented an association between acute parvovirus B19 infection and the subsequent development of fatigue [1••,59–62], CFS [1••,10,63–65], and FM [66] (Table 1). Regarding the diagnosis of B19-associated CFS (in all of the cases except one) [63], the CDC criteria for the diagnosis of CFS were fulfilled. In addition, a recent study on the safety and immunogenicity of a recombinant B19 vaccine revealed short-lived fatigue occurring in eight of 24 healthy volunteers after vaccination [67]. This is interesting because it suggests that virus replication is not necessary for the symptom of fatigue; these recombinant virions lack DNA and are replication-defective.

There have been attempts to identify particular markers that may be useful to indicate that a particular case of

Table 1. Documented cases of fibromyalgia, fatigue, and chronic fatigue syndrome associated with infection or vaccination against parvovirus B19

Study	Clinical details	Cases, *n*	Total cases studied	Clinical diagnostic criteria	Diagnosis of B19 infection	Additional information
Leventhal *et al.* [66]	Fibromyalgia	3	N/A	ACR	IgM+	N/A
Ballou *et al.* [67]	Fatigue	8 (33%)	24	N/A	Post-B19 vaccine	N/A
Hillingso *et al.* [59]	Fatigue	2	N/A	N/A	IgM+/DNA+	LFTs raised
Adlakha and Schultz [60]	Fatigue	1	N/A	N/A	IgM+	N/A
Keonigbauer *et al.* [61]	Fatigue	1	N/A	N/A	IgM+	After transfusion
Kerr *et al.* [1••]	Fatigue	11 (22%)	51	N/A	IgM+	N/A
Hayakawa *et al.* [62]	Fatigue	9 (42%)	21	N/A	IgM+	N/A
Jobanputra *et al.* [63]	CFS	1	N/A	ND	IgM+	N/A
Kerr *et al.* [64]	CFS	2 (4%)	53	CDC	Follow-up	N/A
Jacobsen *et al.* [65]	CFS	1	N/A	CDC	IgM+/DNA+	Responded to IVIG
Kerr *et al.* [1••,2••,71••]	CFS	5 (13%)	39	CDC	Follow-up	Cytokine dysregulation; three patients responded to IVIG
Chia and Chia [10]	CFS	3 (1.5%)	200	CDC	IgM+/DNA+	N/A

ACR—American College of Rheumatology; CDC—Centers for Disease Control; IgM—immunoglobulin M; IVIG—intravenous immunoglobulin; LFT—liver function tests.

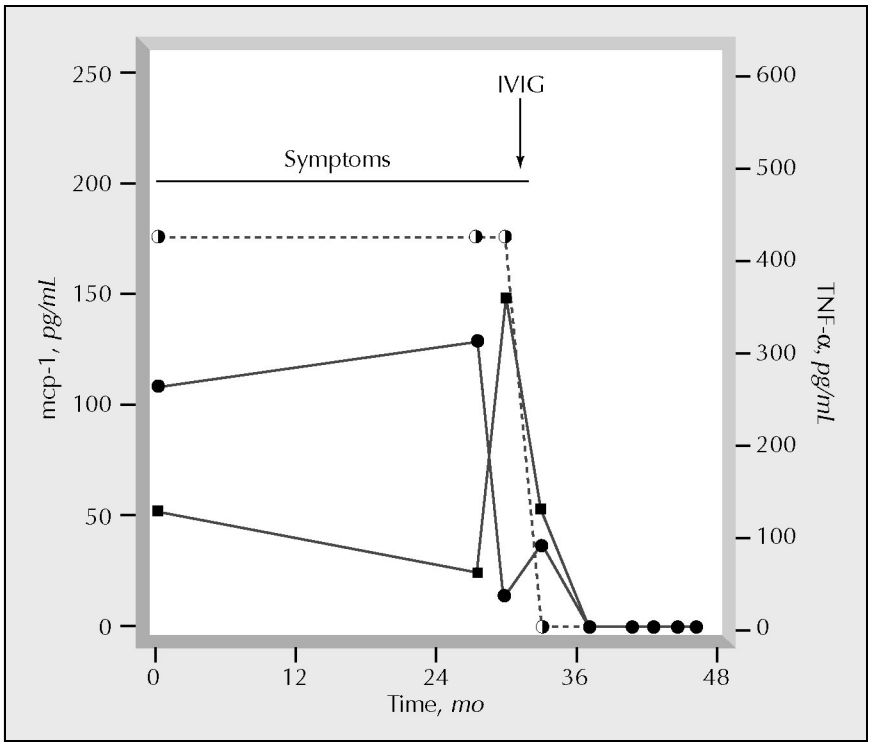

Figure 1. Time course of patient number eight with parvovirus B19-associated chronic fatigue syndrome (CFS) showing duration of clinical symptoms, serum viral DNA (*half-circle*), serum cytokines (TNF-α; *full circle*), and MCP-1 (*square*) before and after the administration of intravenous immunoglobulin (IVIG). *(Modified from* Kerr *et al.* [71••] with permission).

CFS is associated with B19 infection. Although the gold standard method of diagnosis is to have documented acute B19 infection (IgM or DNA-positive) at the time of onset of fatigue and related symptoms, this would be a rare occurrence and a more practical method would be needed to detect serum B19 DNA during the illness [1••].

Four studies have addressed (directly or indirectly) the issue of the proportion of CFS caused by B19 virus, showing various results: 0% (zero of 22) [68], 0.015% (three of 200) [10], 14.3% (one of seven with hematologic abnormalities) [69] to 7.7% (four of 52) [Kerr, unpublished data]. Because several different infections may precipitate CFS [10], the proportion resulting from any one agent, such as parvovirus B19, is likely to vary according to the sampling strategy, time and place, and its relation to the prevalence of each infection. Two particularly important factors are whether there is an outbreak in progress at a particular location and the selection strategy for the control/comparison group.

Parvovirus B19 infection is commonly associated with clinical manifestations of a rheumatic nature and diseases in which pathogenesis, HLA molecules, is known to be important (*eg*, systemic lupus erythematosus) [45,50]. In a recent study, the authors reported that HLA-DRB1*01, *04, and *07 alleles were associated with symptomatic parvovirus B19 infection [70]. Many DRB1*01 and *04 subtypes also encode the shared epitope sequence at position 70 to 74 of the third hypervariable region of the DRβ chain. This forms part of the peptide-binding groove, which is critical in determining which antigenic peptides are presented to T cells and which is a risk factor for the development and severity of RA. This study also documented an association between fatigue occurring during acute B19 infection and the presence of the shared epitope ($P = 0.047$) [70].

Cytokines in parvovirus-associated chronic fatigue syndrome

It has been shown that 13 of 39 cases of acute symptomatic B19 infection had prolonged fatigue that was associated with detectable IFN-γ (≥ 7 pg/mL) or TNF-α (≥ 40 pg/mL; $P = 0.0275$) [1••,2••]. In five of these 13 patients with fatigue, their symptoms fulfilled the CDC criteria for a diagnosis of CFS. Mean circulating IFN-γ and TNF-α in these five CFS cases were 8.37 and 65.71 pg/mL, respectively [2••]. Four of these five cases were viremic at follow-up, suggesting the use of a serum test for B19 DNA in the investigation of patients with CFS.

The authors have treated three cases of B19-associated CFS, which followed acute parvovirus B19 infection with a 5-day course of intravenous immunoglobulin (IVIG) (400 mg/kg/d), the only specific treatment for B19 infection [71••]. During the pretreatment phase of the illness, each patient was viremic and had consistently elevated levels of TNF-α and MCP-1; IFN-γ and IL-6 also were detected at certain time points. IVIG therapy led to clearance of B19 viremia, resolution of symptoms with improvement in physical and functional ability in all of the patients, and resolution of cytokine dysregulation (Fig. 1) [70]. In the patient who was negative for parvovirus-specific IgG, this antibody was detected for the first time after IVIG treatment and its appearance was associated with an isolated increase in IL-2 and IL-4, cytokines that are known to be important in immunoglobulin class switching [72,73]. It has been suggested previously that detectable circulating IL-2 may

Table 2. Circulating cytokines associated with acute parvovirus B19 infection and B19-associated CFS in healthy subjects

	Parovirus B19 infection				
Cytokines	Acute [2••,82••]	B19-CFS [2••,71••]	Normal [2••,82••]	Fibromyalgia [34–36]	Idiopathic CFS [16]
IL-1α	N/A	N/A	N/A	N/A	Conflicting reports; evidence of an effect on the HPA axis, increased prolactin and HGH, reduced thyroglobulin and LH, increased sleep, and reduction in cardiac myocyte contractility
IL-1β (pg/mL)	14.33±38.98	0	0.38±0.3	5.14±0.98	Menstrual cycle-related differences, which likely are secondary to fluctuations in estrogen and progesterone
IL-2 (pg/mL)	2.7±12.01	0	0	N/A	Doubtful relevance
IL-4 (pg/mL)	66.06±0.53	9.56±21.39	2.29±1.71	N/A	N/A
IL-6 (pg/mL)	7.17±18.84	7.89±17.64	0.23±0.11	7.19±0.27	Increased, but inconsistently
IL-8 (pg/mL)	122.43±20.31	28.8±64.40	0.54±0.39	26.3±2.9	N/A
IL-10 (pg/mL)	3.13±15.76	0.8±1.79	0.65±0.51	N/A	Implicated by one study
TNF-α (pg/mL)	271.13±529.25	65.71±28.68	7.59±3.38	N/A	Implicated by several studies
IFN-γ (pg/mL)	25.89±44.54	8.2±10.21	2.34±1.36	N/A	Conflicting reports
MCP-1 (pg/mL)	187.27±24.45	112.57±30.43	10.43±3.68	N/A	N/A
TGF-β1 (ng/mL)	58.28±1.89	57.74±19.26	42.95±2.21	N/A	Implicated in three of four studies
ET-1 (pg/mL)	13.39±9.66	5.53±5.15	0.48±0.12	2.74±0.76	N/A
Neopterin (nmol/L)	4.62±0.26	13.93±11.36*	6.26±0.39	N/A	Conflicting reports; reports of a correlation of an increased level with reduced cognition
β-Microglobulin	N/A	N/A	N/A	N/A	Implicated in three of four studies

*Increased serum neopterin has been associated with asymptomatic parvovirus B19 viraemia [84].
CFS—chronic fatigue syndrome; HGH—high growth hormone; HPA—hypothalamic-pituitary-adrenal; LH—luteinizing hormone.

protect against chronic symptoms after acute parvovirus infection [2••], which has been suggested in studies of B19 infection in the human fetus [74]. To our knowledge, these papers are the only published studies [2••,71••] of cytokines in B19-associated CFS.

Cytokines in acute parvovirus B19 infection

It has been demonstrated that chronic immune activation and cytokine dysregulation occur during and after symptomatic acute parvovirus B19 infection. Cytokine dysregulation has been linked with B19-associated hemophagocytosis and pancytopenia [75], arthritis [56], and myocarditis [76•]. The authors have shown that symptomatic acute parvovirus B19 infection (n = 51) was associated with detectable circulating IL-1β (20%), IL-2 (6%), IL-6 (26%), IL-10 (4%), TNF-α (49%), and IFN-γ (67%) [2••]. Fatigue was associated with IFN-γ ≥ 7 pg/mL in those ≥ 15 years of age (P = 0.022), but not with TNF-α. After a mean period of follow-up of 22.5 months (n = 39), these abnormalities had corrected somewhat; however, a significant number continued to have detectable circulating IL-1β (0%), IL-2 (2%), IL-6 (6%), IL-10 (10%), TNF-α (56%), and IFN-γ (67%) [2••]. It also has been shown that lymphocytes from convalescent adults produced high levels of IL-2 and IFN-γ in response to VP1 and VP2 proteins and that there was a defect in the IFN-γ response to these proteins in recently infected children [77•], which may partly explain why childhood parvovirus infections are asymptomatic more frequently compared with B19 infections that occur later in life. Among the animal parvoviruses, raised levels of serum TNF-α have been associated with enteritis as a result of canine parvovirus [78] and TNF-α and IFN-γ play a role in the pathogenesis of Kilham rat virus-induced autoimmune diabetes mellitus in rats [79].

It also has been shown that parvovirus B19 NS1 protein upregulates IL-6 transcription by binding the NF-κB site in the IL-6 promoter [80], which suggests that the cytokine dysregulation occurring during and after B19 infection may be mediated by the same mechanism. If this is the case, then one would expect that B19-associated cytokine dysregulation also would comprise upregulation of other cytokines, chemokines, immunoreceptors, and cell adhesion molecules in addition to those already mentioned because active NF-kB participates in the control of more than 150 target genes [81].

This hypothesis is supported by documentation of a widespread cytokine and chemokine dysregulation during and after B19 infection [82••].

Conclusions

In the studies of B19, there have been cases that clinically fit diagnostic criteria for CFS (Table 2). The authors have been able to document the clinical and virologic aspects and the circulating cytokines and have shown that, in three cases, these abnormalities and clinical symptoms resolved after therapeutic administration of IVIG. This experience suggests that a promising line of further research would be to prospectively study cases of infection with known agents to document their clinical symptoms, immune responses, and circulating cytokines. It is expected that the particular profile of circulating cytokines would vary with the particular infectious agent and the individual human host. In the setting of rheumatic disease, it would appear that TNF-α is a key molecule in certain diseases and that treatment to reduce it or its effect is beneficial. One report documents that, in three cases of B19-associated autoimmune vasculitis, which were complicated by fatigue, treatment with etanercept (a TNF-α inhibitor) led to a cure [83]. With a better understanding of the microbial etiology and molecular pathogenesis of the CFS, we will be better equipped to design effective treatment strategies that may target the infecting agent or component parts of the immune response.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1.•• Kerr JR, Bracewell J, Laing I, *et al.*: **Chronic fatigue syndrome (CFS) and arthralgia following parvovirus B19 infection.** *J Rheumatol* 2002, **29:**595–602.

This paper documents symptoms during acute and convalescent B19 infection and reports an incidence of CFS after symptomatic infection of 13% (five of 39 cases).

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