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Lower frequency of IL-17F sequence variant (His161Arg) in chronic fatigue syndrome patients

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ABSTRACT

Chronic fatigue syndrome (CFS) is characterized by immune dysfunctions including chronic immune activation, inflammation, and alteration of cytokine profiles. T helper 17 (Th17) cells belong to a recently identified subset of T helper cells, with crucial regulatory function in inflammatory and autoimmune processes. Th17 cells are implicated in allergic inflammation, intestinal diseases, central nervous system inflammation, disorders that may all contribute to the pathophysiology of CFS. IL-17F is one of the pro-inflammatory cytokines secreted by Th17 cells. We investigated the association between CFS and the frequency of rs763780, a C/T genetic polymorphism leading to His161Arg substitution in the IL-17F protein. The His161Arg variant (C allele) antagonizes the pro-inflammatory effects of the wild-type IL-17F. A significantly lower frequency of the C allele was observed in the CFS population, suggesting that the His161Arg variant may confer protection against the disease. These results suggest a role of Th17 cells in the pathogenesis of CFS.

Chronic fatigue syndrome (CFS)/myalgic encephalomyelitis is defined by a severe and debilitating fatigue associated with a variety of symptoms including musculoskeletal pain, sore throat, tender lymph nodes, sleep abnormalities, neurocognitive problems [1]. The pathogenesis of CFS is still poorly understood, but is likely to be multifactorial; viral infections, stress, neuroendocrine dysfunctions, exposure to toxins have all been proposed as contributing factors to the onset and maintenance of the disease [2].

A large body of evidence supports the implication of chronic immune activation and other immunological dysfunctions in CFS. Decreased NK cell activity [3] and dysregulation of interferon pathways [4] have been observed in CFS patients. Increased oxidative stress levels have been detected, which is consistent with a chronic inflammatory situation [5]. Decreased NK cell activity, increased allergic and autoimmune manifestations in CFS suggest a Th2-oriented cytokine pattern [6], a hypothesis supported by the observations that IFN-γ production is decreased, whereas IL-4 production is increased in CFS patients [7–9]. Genetic predispositions may contribute to alterations of cytokine profiles in CFS patients: a first study of cytokine gene polymorphisms found an association between CFS and the frequency of TNF-857 and IFN-γ 874 rare alleles [10].

Another class of cytokines may be relevant in the context of CFS: those associated with T helper 17 (Th17) cell function. Th17 cells have recently been identified as a new subset of T helper cells, in addition to the traditional Th1 and Th2 subsets. Differentiation of Th17 cells from naïve CD4+ T cells requires the coordinate action of the pro-inflammatory cytokine IL-6 (produced by activated macrophages/dendritic cells), and of the immunosuppressive cytokine TGF-β. Differentiated Th17 cells produce IL-17A, IL-17F, IL-22, which appear to mediate host response to specific infections (extra-cellular bacteria), and to play crucial regulatory functions in inflammatory and autoimmune processes [11–14]. A common variant of IL-17F, His161Arg, has been characterized; this variant lacks the ability to activate cytokine production in target cells, and confers protection against the pro-inflammatory effects of wild-type IL-17F [15]. The Arg/His amino acid substitution is determined by a T/C polymorphism in the IL-17F gene (rs763780).

Th17 cells have been implicated in pathologies that share certain symptoms with CFS: inflammatory bowel disease, rheumatoid arthritis, allergic inflammation [16–18]. To investigate the role of Th17 cells, and more specifically of the cytokine IL-17F, in the pathogenesis of CFS, we studied the association between CFS and the frequency of the IL-17F His161Arg variant. The rs763780 polymorphism was analyzed in 89 CFS patients, compared with 56 healthy controls.

Subjects and methods

Eighty-nine CFS patients (40 ± 12 year old) were enrolled in the study. All were diagnosed for CFS according to the clinical criteria

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of Fukuda et al. [1]. Fifty-six aged-matched controls (37 ± 12 year old) were also recruited. Seventy-nine percent of the patients, and 70% of the controls, were females. All subjects were of European origin.

Genomic DNA was extracted from whole blood using the Blood and Tissue DNA extraction kit, Qiagen (Venlo, Netherlands). Analysis of the rs763780 polymorphism was performed with the SNPlex™ genotyping technology, Applied Biosystems (Foster City, CA, USA). Genotyping analyses were performed by DNAvision S.A. (Charleroi, Belgium), using ISO17025-accredited procedures.

Results and discussion

We found a significantly lower prevalence of the His161Arg variant in the CFS population, compared to the control population. Eight out of 89 patients have a CT genotype (8.9%), versus 14 out of 56 controls (25%). None of the CFS patients have the CC genotype, versus 2 out of 56 controls (3.6%). In total, 28.5% of the controls have a CC or CT genotype versus only 8.9% of the patients (OR = 4.05, $p = 0.0018$).

The observed lower frequency suggests an involvement of IL-17F, and more generally of Th17 cells, in the pathophysiology of CFS. The His161Arg variant antagonizes the pro-inflammatory effects of wild-type IL-17F, and thereby exerts a protective effect against asthma [15]. Similarly, we can make the hypothesis that the development and/or maintenance of CFS involves an increased production of IL-17F, and that expression of the inactive variant confers protection against the disease.

Increased IL-17F production has been observed in various inflammatory situations [11,15]. Interestingly, it also occurs in the context of intestinal disease, such as Crohn's disease or ulcerative colitis [19]. Intestinal dysfunction is a common symptom in CFS. Alterations of the intestinal microbial flora have been reported [20]; such alterations can lead to intestinal mucosal dysfunction, increased intestinal permeability (leaky gut), that will finally cause an immune response to the LPS of gram-negative enterobacteria [21,22]. Exposure of immune cells to LPS could therefore be the clue to the chronic immune activation observed in CFS patients. Considering this hypothesis, it is particularly noteworthy that in addition to IL-6 and TGB-β, optimal Th17 cell induction requires the action of Toll-like receptor (TLR)-activated peripheral blood mononuclear cells (PBMCs). TLR-activated monocytes may contribute to Th17 cell induction by cell–cell contact, through ligation of the T-cell receptor [23]; alternatively, TLR-activated PBMCs may secrete a specific set of cytokines that will potentiate Th17 induction [24]. In this last report, it was shown that TLR-4 (receptor for LPS), as well as TLR-7/8, evoked the most robust induction of Th17, whereas stimulation of TLR-1, -2, -3 or -9 was not efficient.

Th17 cell induction could therefore be the link between exposure to enterobacterial LPS and the symptoms of chronic inflammation associated with CFS. Interestingly, Th17 and Th1 responses are mutually exclusive, since IFN-γ suppresses IL-17 and vice-versa [11]. Th17 induction is therefore consistent with a decreased production of Th1 cytokines, as seen in CFS. The pro-inflammatory effects of Th17-secreted cytokines are also consistent with other specific dysfunctions observed in CFS patients: IL-17 and IL-22 can disrupt the blood–brain barrier; Th17 lymphocytes transmigrate across the blood–brain barrier endothelial cells and promote inflammation of the central nervous system [25]. Blood–brain barrier permeability and CNS inflammation is thought to be a key aspect in the pathogenesis of CFS [26].

The implication of Th17 cell activation in the pathogenesis of CFS would be a significant progress in the understanding of the disease, opening new therapeutic perspectives. Confirmation of our results on a larger number of samples is therefore warranted. Further genetic studies will also look at other IL-17F-related genes, such as IL-23R. IL-17F is indeed specifically produced by IL-23R-expressing Th17 cells; IL-23R is also polymorphic and some variants of this receptor predispose to inflammatory bowel disease [16,19]. An association between CFS and polymorphisms of the IL-23R gene, or other functionally related genes, would provide additional support for the implication of intestinal dysfunction and IL-17 axis in the pathogenesis of CFS.

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