Identification of anti-citrullinated protein autoantibodies in CFS

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Introduction

• Chronic Fatigue Syndrome (CSF), recognized as Myalgic Encephalomyelitis (ME) by the World Health Organization, is a devastating neuromune disease that affects over two million Americans [1,2].

• CFS/ME symptoms include a profound post-exertion fatigue and a prolonged metabolic recovery period in response to even modest physical or mental activity. Muscle pain, cognitive difficulties, somatosensory hypersensitivity, poor temperature control, and unrefreshing sleep are associated with the disease.

• Research shows that CFS/ME stems from an autoimmune reaction to a viral, bacterial, or environmental trigger [3].

• The presence of anti-citrullinated protein antibodies in rheumatoid arthritis (RA)[4,5], another autoimmune disease, suggests that the possibility of aberrant citrullination of a protein may underlie the physical and cognitive impairments of CFS/ME patients. Citrullination is an enzymatic process that converts arginine into citrulline, which could trigger an autoimmune reaction.

Hypothesis

It is possible that some system protein in CFS/ME patients becomes abnormally citrullinated in response to a viral infection (or some other foreign agent).

• The immune system, recognizing the citrullinated protein(s) as a foreign object, will generate an antibody (an “autoantibody”). The autoantibody will attack the modified protein, which results in compromised protein function and tissue inflammation.

• No previous studies have reported the presence of an anti-citrullinated protein antibody (ACPAs) in CFS/ME.

• We tested the hypothesis that plasma from blood drawn from CFS/ME patients contains an ACPA that is not present in healthy subjects.

Methods

We used an ELISA based system in which a probe, a cyclic citrullinated peptide (CCP), binds to an anti-citrullinated protein antibody (ACPAs), if present. The system, routinely employed in RA diagnosis, was used to measure the titer of two anti-CCP antibodies, IgG1 and IgG4. A colorimetric assay employed a secondary antibody, pre-conjugated to an enzyme that catalyzes a chromogenic substrate.

Serum from 25 adult patients were analyzed and compared to results from 25 healthy age- and sex-matched control subjects. Blood plasma samples were obtained from a national repository (the Solve CFS BioBank), administered by the Solve CFS/ME Initiative (a national organization dedicated to CFS/ME education and research). Subject information was kept confidential. Fifty 100 microliter aliquots were coded with a unique identifier and sent to Dr. Rincon, who conducted the blinded analysis. The aliquots were used to detect the presence of anti-CCP antibodies in the serum of patients with RA [20].

Results

• Plasma samples from 7 of 25 CFS/ME subjects tested positive for anti-CCP antibody.

• Plasma samples from 2 of 25 control samples tested positive for anti-CCP antibody.

• Of the 25 CFS/ME samples, 2 tested positive for IgG1-anti-CCP antibody, 4 for IgG4-anti-CCP antibody, and one for both.

• Of the 25 control samples, 2 tested positive for IgG4-anti-CCP but none for IgG1-anti-CCP. It is possible that the two who tested positive had RA [6].

Discussion

An anti-citrullinated protein antibody (ACPA) was detected in roughly a third of the ME/CFS patients, advancing the possibility that citrullination of a protein or family of proteins may underlay the physical and cognitive disabilities in some CFS/ME patients. However, this pilot study was limited by the small number of samples tested (50), requiring additional tests with larger sample sets (e.g., 250 or 500 samples) for confirmation and validation. If validated, our results would strongly suggest that a significant subset of CFS/ME patients (perhaps up to a third of the ~2 million Americans with the disease) may have an ACPA-mediated autoimmunity that causes the disease phenotype.

CFS/ME is a systems-wide neuromune disease with metabolic abnormalities that lead to profound fatigue. The precise cause of CFS/ME is not known, but autoimmunity (with genetic susceptibility) is strongly indicated [7]. Gene signatures studies report differences between CFS/ME and healthy subjects, with sensory, adrenergic and immune system receptor expression level elevated in a majority of CFS/ME patients and alpha 2A receptor expression level reduced in the remainder [8,9]. Immune system disturbances have been correlated with altered cytokine number, CD4 cell count, and RNAse L levels [10]. Viral infections such as cytomegalovirus (CMV), herpes virus HHV-6, and particularly Epstein-Barr virus (EBV) have often been associated with CFS/ME [11]. Anti-viral agents such as valacyclovir [12] or valganciclovir [12] have been reported to benefit some patients who are chronically infected with EBV, CMV, or other herpes viruses.

In light of the effect CFS/ME on the brain, central nervous system, and muscle function, our results suggest the possibility that an ACPA targets systemic proteins serving these systems. Proteins of the vasculature and/or mitochondria are prime candidates. It is reasonable to speculate that signaling pathways leading to citrullination of the proteins are triggered by traumatic stress, such as infection with EBV, assisted by a genetic vulnerability.

Conclusions

We plan to repeat this preliminary experiment using a much larger sample set. If our results are corroborated, we will conduct future experiments designed to isolate, identify, and characterize the putative citrullinated protein(s) – initially focusing on the candidates above.

Our results, although preliminary, suggest the possibility that specific isotypes of anti-citrullininated protein antibodies may be applied to blunt the disease pathology in specific subsets of CFS/ME patients. For example, Dr. Rincon has shown recently that tocilizumab (a commercially available biological drug that blocks the IL-6 receptor) selectively reduces the IgG4 type, but not the IgG1 type, of anti-CCP antibodies in RA patients [20].

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References


