Journal of Nutritional & Environmental Medicine (2001) 11, 241–247

CLINICAL RESEARCH

Improved Immune Activation Markers in Chronic Fatigue and Immune Dysfunction Syndrome (CFIDS) Patients Treated with Thymic Protein A

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Abstract

Purpose: *To evaluate the effects of the orally administered thymic protein A on clinical blood parameters and the subjective symptoms common to patients with chronic fatigue and immune dysfunction syndrome (CFIDS).*

Materials and Methods: *A novel immune modulator, thymic protein A, in oral formulation was tested in 23 CFIDS patients manifesting clinical symptoms of CFIDS and abnormal $CD8^+$ subpopulations and interferon pathway-associated enzyme levels.*

Results: *Sixteen of the 23 patients experienced normalization of immune function with a corresponding improvement in clinical symptoms of CFIDS.*

Conclusion: *The data suggest that reinstitution of immune regulation with thymic protein A may ameliorate symptoms associated with CFIDS.*

Keywords: chronic fatigue, immune regulation, thymic protein, oral treatment.

INTRODUCTION

Chronic fatigue and immune dysfunction syndrome (CFIDS) is a complex illness that involves a broad array of symptoms with an unknown etiology. First described in 1980 and defined by the Centers for Disease Control (CDC) in 1994, the diagnosis and treatment of CFIDS remain a mystery. Symptoms of the disease involve a wide range of organ systems including immunological, neurological, and musculoskeletal systems. An increasing body of evidence suggests that the onset of CFIDS is associated with a stressful life event as well as a period of physical stress characterized by overwork, lack of sleep and a poor diet [1, 2]. This, commonly, is followed by a 'flu-like illness, weakness and profound fatigue. This disease affects over 800,000 people in the USA and 50% of these are disabled and cannot work.

The CFIDS syndrome is characterized by unexplained persistent and/or relapsing fatigue that interferes with the ability to carry out normal daily activities. In addition to the fatigue, patients experience the occurrence of four or more of the following symptoms: substantial impairment in short-term memory or concentration; sore throat; tender, swollen lymph nodes; muscle pain; multi-joint pain without joint swelling or redness; headaches of a new

ISSN 1359-0847 print/ISSN 1364-6907 online/01/040241-07 © Taylor & Francis Ltd
DOI: 10.1080/13590840120103085

type, pattern or severity; non-restorative sleep; and post-exertional malaise lasting more than 24 hours. These symptoms must have persisted or recurred during six or more consecutive months of illness and must not have pre-dated the fatigue.

Many of the patients with CFIDS have elevated titers to viruses such as Epstein-Barr, Cytomegalovirus and Human Herpes Virus 6. It is still unclear whether the elevated titers are indicative of active infection, or dysregulation of the immune response [3, 4]. Investigators believe that a virus infection or re-activation may be secondary to an immunological dysregulation [2, 5]. Several abnormal immune parameters have been reported in CFIDS. Increased numbers of activated $CD8^+CD38^+$ T lymphocytes and a decreased natural killer (NK) cell activity are present especially in those afflicted with serious disease. Reports indicate that NK cell activity is elevated in 20% of cases. Patients with CFIDS often have an altered T4/T8 helper/suppressor ratio [4, 6]. It has been suggested that some of the clinical symptoms of CFIDS may be due to cytokines produced by this hyperactive immune response to a virus or persistent antigen that may still be present in the host or that has been eliminated, but leaves behind these abnormal immunological sequelae [6]. In fact, evidence of stimulation of interferons, antiviral and immunoregulatory cytokines in CFIDS is the activation of the 2,5A-synthetase 1/RNAse L enzymes [7].

Over the past 30 years, a number of peptides have been derived from the thymus gland [8]. As the central organ for the control of the immune system, the thymus gland regulates immune function with the production of various T cells. The original demonstration of the importance of the thymus occurred with its removal. Neonatal or adult thymectomy results in a multitude of dysfunctional immunological symptoms that can be partially restored with the administration of crude thymic extracts or purified thymic peptides.

In the past several years, a native protein derived from thymic epithelium has been tested for its ability to restore immune regulation in both animals and humans. Studies have indicated that this thymic protein can induce and enhance immune function [9, 10]. More recently, this fully intact native biomolecule (50 kDa) has been demonstrated to be active when taken orally as a sublingual powder. It has been known for almost a century that oral administration of large molecules, e.g. egg ovalbumin, can induce specific systemic immune tolerance [11]. Furthermore, studies confirm that large intact proteins cross mucous membrane barriers intact and alter the response of the systemic immune system [12]. It has most recently been shown that intact proteins can be absorbed across mucous membranes [13]. Sublingual administration of grass pollen antigens has been demonstrated to reduce allergic responses. Further evidence of the exquisite specificity of the absorption is that a single amino acid change in a protein can ablate the response [14]. The current study was performed using a sublingual thymic polypeptide on a group of patients who met the diagnostic criteria for CFIDS. The purpose of the study was to evaluate the effects of the orally administered thymic protein A on clinical blood parameters and the subjective symptoms common to patients with CFIDS.

MATERIALS AND METHODS

A group of 36 patients was identified as fulfilling the criteria for CFIDS in that they had been ill for at least 1 year, had been treated for 1 year, and their symptoms had become permanent and stationary. Each patient was asked to sign an informed consent and agreed to participate in a 3-month trial of the oral sublingual nutritional supplement, Longevity Science thymic protein A (see Acknowledgements). A physician saw the patients at the initial visit, at 6 weeks and on completion of the study. A physical examination was performed and blood was drawn for baseline evaluations of NK cell numbers and activity, total white blood cell count, T cell subsets including $CD8^+CD11b^+$, $CD8^+CD38^+$ and $CD8^+HLADR^+$. Levels of interferon-induced protein were measured via the 2,5A-synthetase and RNAse L cleavage activity pathways. These laboratory studies were

TABLE 1. Peripheral blood cell changes

Subpopulation	Mean pre Rx ± SD	Mean post Rx ± SD	Results	p value
Total white blood cells	5591 ± 1129	6100 ± 1500	Increase	< 0.01
$CD8^+$, $11b^+$	25.9 ± 14.1	36.3 ± 24.2	Increase	< 0.03
$CD8^+$, $CD38^+$	113.5 ± 44.4	90.3 ± 45.3	Decrease	< 0.02
$CD8^+$, HLA DR^+	30.61 ± 18.11	20.26 ± 9.29	Decrease	< 0.02

SD, standard deviation. Rx, treatment.

performed at Immunosciences Laboratory (Los Angeles, CA, USA) according to previously published methods [15] and the data were analyzed using a paired two-sample Student's *t*-test. This *t*-test form does not assume that the variations of both populations are equal. It is used when there is a natural pairing of observations in the samples, such as individuals being tested before and after treatment.

A sufficient supply of thymic protein A was given to each patient at a dosage of three packets daily for 90 days. Each packet contained 4μg of purified thymic polypetide concentrate. The patients were instructed to keep a diary of their dosage schedule to track the time and the date that each supplement was taken and to report their responses to treatment.

Each subject was given a questionnaire describing the various symptoms they experienced on a regular basis. The questionnaire had six primary categories: energy; cognitive; psychological; nervous system; immune system, e.g. swollen lymph nodes; miscellaneous. The participants were asked to give a score of 1–5 for the frequency and intensity of the symptoms listed. The symptom questionnaire was given following the completion of the study.

The patients were asked to keep a calendar of the doses taken each day. Each patient completed a questionnaire at the end of each month. The questionnaire asked the patients to rate on a scale from 1 to 5 both the intensity and the frequency of the symptoms common to patients with CFIDS.

RESULTS

Of the initial 36 patients enrolled, 23 patients completed the study. Thirteen patients dropped out due to apparent unpleasant effects. It is well known that many immune modulators, particularly interleukin-2 (IL-2), can cause mild 'flu-like symptoms. Because thymic protein A induces IL-2 production [9], patients suffering from CFIDS, who may be hypervigilant about their condition, could perceive an initial exacerbation of symptoms.

Of the 23 patients who completed the study, there was an overall significant ($p < 0.01$) increase in total white blood cell counts (Table 1). Sixteen of the 23 patients (70%) manifested this increase, despite the fact that only one patient had a below normal count. In fact, the levels (absolute counts) of $CD8^+CD11b^+$ (T suppressor cell phenotype) showed a mean increase from 25.9 to 36.3 ($p < 0.03$; Table 1). This T cell, which decreases with CFIDS, is one of the major diagnostic markers. In the 23 patients who had measurable levels of $CD8^+CD11b^+$, 15 of them (65%) experienced an increase in this key marker. Of the 15, eight had an increase of 50% or more. Six of these improved by 100% or more. The two patients with the greatest increases reported the greatest overall benefit.

Reciprocally, $CD8^+CD38^+$ and $CD8^+HLADR^+$, known activation markers for CD8 cells, showed a statistically significant decrease in their numbers with use of thymic protein A (Table 1). Seventeen of the 23 patients (74%) had a decrease in $CD38^+$ cells and 14 of 23 (61%) had a decrease in $HLADR^+$ cells. Twelve of 23 (54%) had a coordinate response

TABLE 2. Natural killer cells and activity

Subpopulation	Mean pre Rx ± SD	Mean post Rx ± SD	Results	*p* value
$NKHI^+T3^-$	209.57 ± 72.66	219.74 ± 101.27	Increase	< 0.33
NK cell activity	20.70 ± 9.3	21.50 ± 18.8	Increase	< 0.81*

NK cell, natural killer cell; SD, standard deviation.
* Not significant.

of the CD8 subpopulations, thus when the $CD8^+11b^+$ cells went up the activated $CD38^+$ and $HLADR^+$ subpopulations went down.

The remaining immune-related parameters observed were NK cell numbers and NK cell activity. Although both absolute numbers and activity were increased, the results were not statistically significant (Table 2). This result is not totally unexpected, as thymic protein A does not directly affect NK cells. It would only have an indirect effect via its known stimulation of CD4 lymphocytes. Furthermore, recent evidence suggests that NK cells are activated via receptors recognizing glycosyl residues [16].

Of the six patients with abnormal RNAse L and 2,5A-synthetase, four of them normalized (Table 3). The other two improved. Patients with normal levels of these enzymes had lowered levels. The overall mean was a decrease from 1.3 to 0.8 of the 2,5A-synthetase ($p < 0.02$) and the RNAse L cleavage enzyme from 9.0 to 7.3 ($p < 0.01$).

There was marked improvement in many of the clinical symptoms associated with CFIDS (Table 4). Many more symptoms were listed on the patient questionnaire, but the ones illustrated were the most dramatically changed. Symptoms decreased in their intensity as well as their frequency. Patients experienced improvement in quality of sleep, anxiety, depression, and panic reactions, symptoms not normally associated with a viral syndrome.

DISCUSSION

The cause of CFIDS remains controversial and unknown [17]. Several viruses, including Epstein-Barr, Herpes Virus 6 and Cytomegalovirus, have been suggested as possible causes. Other researchers have found a link between Mycoplasma infections [18, 19], Lyme disease and CFIDS. However, one common link is the change in specific types of T cell subpopulations. These shifts have been considered indications of immune dysfunction, and occur in many patients with symptoms of CFIDS [4].

Although this study represents a small population of patients, the results of the pre-treatment and post-treatment comparisons were significant. Increases in white blood cell counts were statistically significant. In previous studies of cats with feline acquired immune deficiency syndrome (AIDS) a similar observation was made (unpublished), the increase attributable to the lymphocyte population. An increase in $CD8^+CD11b^+$ cells was also significant at the $p \leqslant 0.03$ level. The latter is indicative of a population of suppressor cells that is decreased in CFIDS and associated with a persistent immune activation state [20]. A concomitant decrease in $CD8^+CD38^+$ and $CD8^+HLADR^+$ activation markers was ob-

TABLE 3. Reduction of interferon activation pathway enzymes

Enzyme	Mean pre Rx ± SD	Mean post Rx ± SD	Results	*p* value
2,5A-synthetase	1.3 ± 0.9	0.8 ± 0.6	Decrease	< 0.002
RNAse L cleavage	9.1 ± 3.3	7.3 ± 2.6	Decrease	< 0.01

SD, standard deviation.

TABLE 4. Improvement in CFIDS symptoms

Symptoms	Intensity*	Frequency**
Non-restorative sleep	9/19 (47%)	10/19 (53%)
Food sensitivities	6/14 (43%)	9/18 (50%)
Chemical sensitivities	8/15 (53%)	7/18 (38%)
Poor short-term memory	8/17 (47%)	8/20 (40%)
Frequent sore throat	6/11 (55%)	5/14 (36%)
Swollen lymph glands	5/12 (42%)	4/15 (27%)
Nasal allergies	8/14 (57%)	9/18 (50%)
Depression	11/14 (79%)	10/17 (59%)
Muscle weakness	10/17 (59%)	9/22 (41%)
Anxiety	5/11 (45%)	11/17 (68%)
Panic reaction	6/6 (100%)	3/5 (60%)
Fatigue worse with exercise	8/23 (35%)	5/23 (57%)
Canker sores	4/5 (80%)	5/7 (71%)

* Percentage of patients who initially reported a score of 3, 4 or 5 for severity of a symptom and at the end of the survey reported a lower score by 1 or more.
** Percentage of patients who initially reported a score of 2 or more for frequency of a symptom and at the end of the survey reported a lower score by 1 or more.

served. The latter would suggest a normalization of the immune status and a reduction in T cell activation.

Thymic protein A is known to augment helper T cell (CD4) function [9, 10]. This could explain the re-establishment of the immune balance. CD4 cells, both Th1 and Th2 subpopulations, coordinate immune responsiveness by producing the right balance of cytokines (IL-2, gamma interferon, IL-4, IL-10). If CD4 cells are reduced, as they can be in viral infections, especially herpes virus, CD8 dysfunction occurs [21]. CD8 function is dependent upon the CD4 helper T cell subpopulation, particularly Th1 cells. The latter produce IL-2, which is important in stimulating CD8 cytotoxic T cells against viruses. Enhancement of Th1, CD4 function may allow the restoration of immune regulation by providing more IL-2 secretion for optimal cytotoxic CD8 immune reactivity.

Another possibility is that helper T cell stimulation has resolved chronic viral infection, which is suggested in CFIDS, although no etiological agent has been identified. In a preliminary study of thymic protein A with six patients with chronically elevated Epstein-Barr virus early antigen antibody titers, four patients showed a significant decrease in titers [22]. The associated decrease in interferon pathway enzymes 2,5A-synthetase and RNAse L would also suggest a reduction in a chronic viral process or other CFIDS causes.

The other significant change in CFIDS is the elevation of two enzymes, 2,5A-synthetase and RNAse L. These enzymes act in one antiviral pathway and are indicative of interferon-mediated immune activation, which is suggestive of viral infection. It has been hypothesized that many of the clinical symptoms are induced by immune activation from viral infections [23]. In the present study, patients taking the thymic protein A had significantly decreased levels of these enzymes. Symptoms relating to CFIDS, such as muscle weakness and fatigue after exercise, improved concomitantly.

Many different treatments have been offered to help patients with debilitating illness. Antibiotics have been useful when the infection is of bacterial origin. Antiviral drugs such as acyclovir and related compounds work in some CFIDS patients [24].

CONCLUSION

In summary, in this study, 16 of the 23 patients experienced improvements in symptoms of CFIDS. Objective measurements of several blood parameters associated with CFIDS

showed statistically significant improvement as well. Because there is no truly effective treatment for patients with chronic fatigue syndrome, the use of thymic protein A may have beneficial effects on the course and severity of the illness. Until recently, the idea of oral administration of proteins has been excluded because of the dogma that large polypeptides would be digested and unabsorbed. Because the product is taken sublingually, the degradation of the protein in the stomach is avoided. Furthermore, large proteins can be absorbed intact across oral mucous membranes [13]. Expanded studies with double-blind, placebo controls will be necessary to evaluate further the effects of this immune regulatory protein.

ACKNOWLEDGEMENTS

Genicel Inc. and Longevity Science provided funding for this study. The authors had no financial affiliation with either of these organizations.

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