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The National CFIDS Foundation, Inc. has announced that the following article and investigative report has been placed in the Fall 2003 issue of "The National Forum" newsletter:

Part One - Unmasked Research:

*STAT1-alpha and p53 Deficiencies are Found in Patients with Chronic Fatigue Syndrome;
The National CFIDS Foundation Responds by Funding Several New Research Initiatives*

Part Two - Total Exposure:

*Expanded Model for RNase L Fragmentation in CFS Uncovered;
The National CFIDS Foundation Announces the Use of Elastase Inhibitors as a Potential
Treatment for CFS Patients*

Accompanying this article are several others directly relating to this breaking news! Please refer to this issue for other pertinent information. If you do not already subscribe to the newsletter, please consider doing so by phoning or writing to the National CFIDS Foundation.

For Better Health!
Gail Kansky,
President

**Part One - Unmasked Research:
STAT1-alpha and p53 Deficiencies are Found in Patients with Chronic Fatigue Syndrome;
The National CFIDS Foundation Responds by Funding Several New Research Initiatives**

Investigative Report by Alan Cocchetto, Medical Director
National CFIDS Foundation, Inc.

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Through its own extensive six month medical investigation, the National CFIDS Foundation (NCF) uncovered research that will have widespread ramifications among the CFS patient community, their physicians, as well as CFS researchers worldwide establishing CFS as a serious and perhaps fatal illness. Because of these implications, the NCF has responded to this major discovery by dynamically funding new medical research by several noted scientists.

Briefly stated, the NCF has uncovered a key scientific discovery, previously made by prominent CFS researchers, that has yet to see the 'light of day.' The critical component: STAT1. The STAT1 protein is crucial for proper immune function and regulation. Without it, cells are unresponsive to interferons leaving the body defenseless against viral and bacterial infections. In fact, human STAT1 deficiency is lethal. In this report, the NCF identified CFS researchers that previously found STAT1 to be absent in CFS patient blood.

"The NCF began its investigation by thoroughly reviewing work that had been previously published by Kenny DeMeirleir, MD, PhD and his colleague's group in Belgium" stated Gail Kansky, President of the NCF. "Then one discovery lead to another and our own investigation just skyrocketed" said Kansky. "As the CFS patient community is aware, Dr. DeMeirleir is a highly visible CFS researcher, both in the U.S. and abroad. He is a medical editor for the Journal of Chronic Fatigue Syndrome, is on the board of the American Association for Chronic Fatigue Syndrome (AACFS), and is actively involved with R.E.D. Laboratories which has worked to further the studies of Dr. Robert Suhadolnik, from Temple University, whose research group made the initial RNase L discoveries in CFS patients" stated Kansky. "I strongly feel that the NCF's investigational and medical research efforts will greatly add to those efforts that we have previously undertaken to unravel the mysteries of CFS. This should lead to much greater understanding of the disease process" said Kansky. Furthermore, she stated that "Our funded medical research is aimed to solidify and scientifically expand this important work so that desperately needed and appropriate patient treatments will follow. The NCF's efforts will continue to illuminate the darkness that has overshadowed this very serious illness."

The NCF first began its investigation with a published book titled "*Chronic Fatigue Syndrome: A Biological Approach*" edited by Dr. Patrick Englebienne and Dr. Kenny DeMeirleir [1]. Published in 2002, examination of the chapters, by NCF staffers, revealed that Dr. DeMeirleir and his colleagues carefully and methodically probed various signal transduction pathways in patients with CFS via examination of their peripheral blood mononuclear cells (PBMC's or monocytes). These authors stated that "*the importance of STAT1 in mediating the action of both type I and II IFNs (interferons) is no longer questioned, as a lack of its expression is consistently associated with IFN resistance. The apparent dysregulation in types I and II IFN pathways in chronic fatigue syndrome led us to investigate the expression of STAT1 in PBMCs. We classified the samples according to the ratio of 37- over 80-kDa RNase L which is representative of the proteolytic activity of the PBMC samples. As shown in Figure 5.9, **STAT1 is fully degraded in positive samples**, suggesting that it may also be a substrate of the proteases responsible for RNase L cleavage. **A degradation of STAT1 in those cells might well constitute the missing link explaining unresponsiveness to IFNs**" (interferons) [2].*

In this figure from the book, with RNase L ratios (37 kDa/80 kDa) greater than 0.25, native STAT1-alpha is lost but some cleavage (breakdown) products are shown until the RNase L ratio hits approximately 2.0. After that, there is the complete absence of both STAT1-alpha and the cleavage product!

Explaining further, STAT is notation for Signal Transducers and Activators of Transcription. STAT's are a family of transcription factors that play central roles in the responses of cells to cytokines, molecules that control every aspect of the immune system [3]. STAT1 has two forms, alpha and beta. **Dr. DeMeirleir's group tested STAT1-alpha, a native 91 kDa protein, in patients with CFS and correlated it with the RNase L ratios.** STAT1-alpha is intimately involved in the response of cells to type I (alpha and beta) and type II (gamma) interferons. Most important is the fact that a **STAT1-alpha deficiency is associated with fatal infections by both viruses and bacteria** [3,4,5,6,7,8]. **Furthermore, in animals, STAT1-alpha deficiency is associated with impaired responses to interferons, increased susceptibility to tumors, as well as impaired growth control. STAT1-alpha also plays a critically important role in antigen presentation.**

The NCF staff then wondered if Dr. DeMeirleir and his colleagues realized the importance, as well as the significance, of their discovery and when might they have first identified it? To answer this question, we uncovered a patent filed in April 1999 titled "*Methods and compositions for use in characterizing multiple sclerosis disease activity in a subject.*" [9] In this patent, Dr. DeMeirleir and his colleagues at R.E.D. Laboratories determined the amount of 37 kDa and 80 kDa RNase L proteins and utilized RNase L ratios to characterize multiple sclerosis disease activity. This patent was filed after one filed by inventor Dr. Robert Suhadolnik, in April 1999, titled "*Chronic fatigue syndrome diagnosis*" in which the diagnosis of CFS was made through the detection and determination of both the 37 kDa and 80 kDa RNase L proteins [10]. Examination of both these patents indicated that, in both CFS and MS, these same RNase L proteins can be used to assist in diagnosis as well as for determining disease activity. Multiple sclerosis patients have previously been found to have alterations in STAT1-alpha [11]. Furthermore, in CFS, this is vitally important due to the correlations that were found between the RNase L ratios and STAT1-alpha.

This is further stated in two additional patent applications by Dr. DeMeirleir's colleagues. One of these applications directly discusses the specific role of STAT1-alpha in CFS patients [12,13]. Quoting these patent applications directly: "***STAT1 plays an important role in growth arrest, in promoting apoptosis and is implicated as a tumor suppressor. STAT1 null cells are resistant to apoptotic induction by TNF-alpha....STAT1 deficient mice exhibit a severe defect in IFN-dependent immune responses against viruses and microbial pathogens....If STAT1 is disabled or otherwise inactive in the cells of the immune system, treatment with interferon or interferon inducer will not be effective in promoting and establishing the interferon-inducible antiviral and antiproliferative pathways.***" Furthermore these applications state "***the results demonstrate that the presence and amount of STAT1 protein fragmentation directly correlates with the presence and amount of low molecular weight RNase L fragments in PBMC samples. These data indicate that native STAT1 protein is fragmented at an earlier point in the disease cycle than RNase L, and that by the time native RNase L is demonstrably attacked by proteases (ratio > 2.0), that native STAT1 protein has entirely disappeared due to proteolysis, leaving the cells unable to respond to interferons and/or interferon inducers.***"

It became obvious to NCF staffers that Dr. DeMeirleir and his colleagues had made a vitally important discovery but had only published part of the information in the book and the remainder in patents or patent applications that we were aware of. Was there other information to be found?

A quick check of the R.E.D. Laboratories website [14] indicated that "*The company's scientists have discovered a number of other cellular proteins that play important roles in the*

*initiation, progression, and pathogenesis of immune dysfunction. **The most important revelation to date has been the discovery of one protein, which is used as a serum-based assay for the detection of chronic immune disease. Such a serum-based marker has been developed as a screening test for the blood supply to reduce the number of transfusion-related infections with persistent (and covert) viral infections.***

It became obvious to the NCF staffers, that both the book and the patent information told a significant story regarding the critical importance of STAT1-alpha in CFS patient blood. **One thing is certain, blood doesn't lie!** Likewise, the true implications, pathological and perhaps life threatening for patients as well as financial potentially for R.E.D. Laboratories, regarding transfusion-related infections in the blood supply by "covert infections", were enormous and certainly worthy of worldwide attention!

Probing further, the NCF discovered vitally important testimony given directly by Dr. Kenny DeMeirleir before the Flemish Parliament of Belgium in a hearing on March 5, 2001 [15]. **In his testimony given before Flemish Parliament, Dr. DeMeirleir stated "Caspases and calpain are induced by cellular stress, which leads to apoptosis. In an intracellular disorder, calcium influx is increased. Calcium will further activate calpain, so that some caspases are inhibited and therefore block apoptosis. One of the cellular proteins that are split by these enzymes is STAT1, the carrier of the interferon-gamma signal in the immune cells, which leads to the Th1-to-Th2 shift. Unfortunately, the Chronic Fatigue Syndrome is often called psychosomatic. This is, however, more indicative of medicine's inability to deal with it. We now understand the nature of the disorder. In its early phase, apoptosis increases. In a subsequent evolution, apoptosis is blocked and the interferon signal disappears due to the destruction of the protein which transports the intracellular interferon signal to the nucleus (where apoptosis is initiated). This leads to more and more infections. This process goes on at all levels (in the central nervous system, the muscle cells, the white blood cells, and so on). Some patients suffering from the Chronic Fatigue Syndrome develop epilepsy. We find that most patients have a light form of epilepsy. This leads to sleep disturbances and a situation where the fatigue increases since one does not recover anymore."**

"Given the true significance of this work and its implication in a functionally oppressive disease that desperately struggles for proper recognition, validation, and affirmation at all levels of medical science, the NCF's Board of Directors unanimously voted to immediately fund several important research grant projects to be carried out by noted researchers" said Kansky.

The NCF first contacted a well-known research group, who has asked to remain anonymous at this time, and arranged to send grant funding to them via our NCF Research Grant Program. "We are very excited by this since this group will be the first to begin studying *"STAT1-alpha in Chronic Fatigue Syndrome Patients"* stated Kansky. Lead scientists for this research group commented that this was an enormous step forward towards understanding the disease process in these patients.

Next, the NCF arranged another research project, also funded by our Research Grant Program. The NCF sent grant money to Dr. Konstance Knox and Dr. Donald Carrigan, both from the Institute for Viral Pathogenesis, for *"A Study for the Potential Role of STAT1 in the Pathogenesis of Chronic Fatigue Syndrome."* "This grant is aimed at significantly expanding our knowledge about the specifics of STAT1 and its role in CFS from a pathological standpoint" stated Kansky. "Furthermore, since we are expecting both teams (first group and Knox/Carrigan) to complete their work with research physicians in the months ahead, we are anticipating significant medical breakthroughs as the direct result of these grants. The NCF has expedited this work because our patients are seriously ill and we anticipate that these efforts will certainly confirm this" said Kansky.

The NCF staff also contacted two world renowned experts on the STAT1 protein: Dr. Joan Durbin from Columbus Children's Research Institute and Dr. David Levy from New York University School of Medicine. Both Dr. Durbin and Dr. Levy have published extensively on STAT1 and are responsible for the development of the animal model for STAT1-alpha deficiencies. In NCF interviews with both of them and from their numerous published medical journal articles, it is clear that the complete loss of the native STAT1 protein constitutes a serious illness that may ultimately be fatal unless this protein defect can be corrected.

Ironically, this is where the NCF is truly hopeful. In one of the patent applications [13], Dr. DeMeirleir's colleagues determined that the *"STAT1 protein is degraded when a cell extract from a healthy control (i.e., 'negative extract'; RNase L ratio < 0.2, STAT1 protein containing) is incubated with a cell extract from a patient (RNase L ratio = 3.0; STAT1 protein negative). This degradation is inhibited in the presence of proteasomal inhibitor (MG132) but not in the presence of the other protease inhibitors tested. Thus the degradation of STAT1 protein is a specific cellular process that involves proteasome and does not involve the apoptotic enzymes caspase-3 or calpain."*

What this implies is that **Dr. DeMeirleir's colleagues had already found a very specific inhibitor, MG132, for STAT1-alpha degradation in CFS patient blood in-vitro!** In our own research, the NCF found that MG132 functions as both a specific inhibitor of the ubiquitin-proteasome pathway [16] as well as a specific beta-secretase inhibitor [17]. Both of these pathways are intimately involved with amyloid formation. Amyloidogenesis is a general phenomenon of protein misfolding. Protein misfolding and the formation of abnormal protein fibrils appear to play a central role in a variety of neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease, etc. This is why the expression of the Huntington's protein in CFS patients, by the CDC using gene expression analysis, is intriguing [18]. These are all scientific clues to a bigger picture for this disease.

Because of our previous efforts with the ciguatera epitope and its effects on neuroblastoma cells as well as other specific unannounced scientific findings related to this epitope, the NCF has also provided new research grant funding to Dr. Yoshitsugi Hokama, from the University of Hawaii, for the *"Development of an Immunological Assay for Assessment of Amyloids in Chronic Fatigue Syndrome."* "Dr. Hokama's previous research dovetailed nicely into the scientific work we are describing here" said Kansky. "In fact, **the NCF believes there is a relationship between the ciguatera epitope, protein misfolding, and amyloid formation.** Amyloids directly alter sodium channels and that is part of the significance of the ciguatera epitope finding as it relates to our previous CFS patient studies. This is something that we had known prior to the initial funding of Dr. Hokama's work but this previous work had to be scientifically verified and expanded upon first before we pressed on further" stated Kansky. The NCF is also aware that a relationship already exists between amyloid formation and STAT1-alpha. Amyloids can potentially bind the STAT1-alpha protein and take it out of commission. This mechanism is very important. Dr. DeMeirleir and his colleague's discovery on STAT1-alpha and RNase L cleavage is central to our working hypothesis but the NCF believes that we have found other missing pieces, regarding the infectious agent as well as other disease mechanisms, that are absent from their work. What is exciting is that **our NCF staff has already identified very specific PCR binding sequences for STAT1-alpha in the infectious agent that we believe is directly responsible for this disease.** This implies that once infection begins, STAT1-alpha becomes one of the first critical immune system proteins to get "chewed up" as a result of being a direct target of the infectious agent thereby greatly damaging the immune system! This would be in agreement with Dr. DeMeirleir's findings where STAT1-alpha binding occurs before the degradation of the native RNase L protein. **"Furthermore, in full agreement with Dr. Elaine DeFreitas' previous work, the NCF believes this infectious agent is of non-human origin"** stated Kansky. "We are meeting the dragon head-on and we hope that our funded research will help crack the code, once and for all, for this disease. The NCF continues to break important new ground in the understanding of the disease mechanisms while going scientifically where

no other CFS organization has ever been before! This appears to be our forte and it reflects our true passion and full commitment to the CFS patient community. As a result, we are much further ahead in the discovery process than most patients and researchers realize" said Kansky.

In addition, **Dr. DeMeirleir's colleagues also made another significant discovery critical to the health and welfare of patients with CFS** [19,20]. Excerpts from the book and the patent application state *"Fig. 1 represents a densitometric scan of a Western blot detecting p53 protein and p53 protein fragments from PBMC extracts from CFS patients....**The above results demonstrate that the presence and amount of p53 protein fragmentation directly correlates with the presence and amount of low molecular weight RNase L fragments in PBMC samples. These data indicate that native p53 protein is fragmented at a later point in the disease cycle than RNase L protein. The loss of functional p53 protein in PBMCs render these cells unable to respond to normal growth inhibitory stimuli and provide the means whereby unregulated cell growth occurs, ultimately giving rise to hematopoietic tumors.**"*

Furthermore, their explanation in this application states: *"Another important protein that regulates the induction of apoptosis is p53. The p53 protein is normally activated in response to genetic damage within the cell and its activation is accompanied by self-stabilization, allowing it to accumulate to high levels and cause cell cycle arrest and induce apoptosis. In addition, the **p53 protein has a critical role in protecting the cell from malignant development**; mutations in the p53 gene (and protein) are the most frequently detected genetic event in cancer. Mutations in p53 may occur at the genetic level (i.e. DNA sequence alterations that change the amino acid structure of the protein), or its function may be altered by alterations in the numerous proteins with which p53 interacts. p53 may also be altered by the action of certain proteases, resulting in cleavage, preventing the formation of active tetramers of the protein. **If p53 is cleaved and/or otherwise disabled in the cells of the immune system, these cells would be blocked from entering the apoptotic pathway if infected with a virus or other microorganism. In addition, persistent inactivation of the p53 protein may lead to increased incidence of cancer** (Levine et. al., *J. Chronic Fatigue Syndrome* 7: 29-38, 2000). Activation of the 2-5A synthetase / RNase L antiviral pathway has been demonstrated to induce apoptosis, while induction of the same pathway in cells expressing mutant forms of p53 was demonstrated to suppress the apoptotic pathway. **The inactivation of p53, RNase L, and other proteins within the cells of the immune system most certainly leads to a dysfunctional immune system, unable to respond to challenge by microorganisms and/or the presence of pre-malignant cells. Indeed the immune system itself may be in a pre-malignant state.**"*

In a review of the literature on STAT1-alpha and p53, we found an article [21] on STAT1 deficient mice that states ***"when the STAT1 deficiency was placed on a p53 null background there were increased rates of tumor formation and an increase in the non-lymphoid tumor types."*** This helps to explain the importance of these two proteins that are essential to appropriate immune responses since STAT1 and interferon are central to antitumor effects [22]. In fact, this study suggests that NK (Natural Killer) cells from STAT1 deficient mice may have a reduced capacity to eliminate tumor cells in-vivo.

Part one article review at a glance:

Dr. DeMeirleir and colleagues scientific finding:

- * STAT1-alpha deficiency in CFS patients
- * p53 deficiency in CFS patients

Scientific interpretation:

- * Loss of STAT1-alpha constitutes a serious illness that may ultimately be fatal because the cells are unresponsive to interferon leaving them unable to adequately defend against infections.
- * Loss of p53 constitutes a pre-malignant state because surveillance against DNA mutations, protein alterations, and unregulated cell growth/division are left unguarded.
- * Loss of these proteins assists in immune deficiency and dysregulation.

**Part Two - Total Exposure:
Expanded Model for RNase L Fragmentation in CFS Uncovered;
The National CFIDS Foundation Announces the Use of
Elastase Inhibitors as a Potential Treatment for CFS Patients**

Investigative Report by Alan Cocchetto, Medical Director

National CFIDS Foundation, Inc.

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From our investigation, the NCF had also identified several additional patents that yielded new information that hadn't been seen by other scientists or physicians in the field. In one patent [23], Dr. DeMeirleir and his colleagues expanded the RNase L fragmentation model in CFS and provided descriptions for their scientific discoveries: ***"The lower molecular weight fragments of RNase L are produced from the specific cleavage of native RNase L by a protease(s). These fragments then play a selective role in inhibiting the apoptotic pathway, in effect keeping the damaged cell alive and the immune system dysfunctional."*** The researchers had identified three RNase L fragments. In Fragment #1, they found an ankyrin binding repeat domain which is known to interact with various transport proteins. In addition, they also identified sequence homology with NF-kappaB (Nuclear transcription Factor kappa B). NF-kappaB has been demonstrated to induce transcriptional activities within a cell to promote cell growth thus acting in an anti-apoptotic manner. In Fragment #2, they identified the 2-5A binding fragment that has catalytic activity and thus is able to degrade RNA. Since this fragment competes with the native RNase L protein for free 2-5A, it may be responsible for inhibiting the complete induction of apoptosis. They found that Fragment #3 shared homology with chain A of Cdk6 (Cyclin dependent kinase). Cdk6 acts to block apoptosis by altering the cell cycle. In fact, the inventors provide a diagram (Figure 1) that explicitly shows the role of these RNase L fragments in CFS pathogenesis. Since the 2-5A synthetase / RNase L pathway is part of the antiviral defense mechanism in cells and because it plays a role in the regulation of cell growth / differentiation, these RNase L fragments provide critically important insight into the cellular mechanisms that are uniquely responsible for the immune dysfunction in CFS patients.

Furthermore, the inventors conclude that *"The presence (or absence) of low molecular weight RNase L fragments 1 to 3, and optionally the caspase activity data, is then used to diagnose whether or not the host suffers from the chronic immune disease."* Caspases are cysteine-aspartic acid proteases that are considered to be the cell death executioners due to the absolute requirements for their presence in cell death / apoptosis [24].

Extensive analysis was done by Dr. DeMeirleir and his colleagues in this patent. They studied four patients who had been administered amplitgen for a period of at least six months. These patients were also compared with healthy controls and forty-four additional patients who had not undergone amplitgen therapy. All patients were evaluated for RNase L fragmentation and caspase levels. For the 44 patients, when the RNase L ratios were 6 to 8 ($6 < \text{RNase L ratio} < 8$), the caspases C2, C3, C6, C8, and C9 reached their peak. Caspase levels were generally lower overall for lower RNase L levels (RNase L ratio < 6) as well as for higher RNase L levels (RNase L ratio > 8). In fact the inventors concluded *"Upon further analysis, it is evident that levels of all caspases assayed (C2, C3, C6, C8, and C9) at first increase, then decrease relative to the RNase L ratio in CFS patient groups P1 through P7, indicating that the apoptotic process is being inhibited when the levels of RNase L-related fragments reaches a certain point."* In their evaluation of four patients treated with amplitgen, two patients responded to therapy in that their RNase L ratio decreased and they experienced increases in their Karnofsky performance scores (KPS). The Karnofsky performance test measures an individual's ability to function and carry on normal activities. Karnofsky scores range from zero for a nonfunctional or

dead patient to 100 for a completely normal person. The third patient did not respond to amplitgen due to an increase in the RNase L ratio and a decrease in Karnofsky score. The last patient responded to amplitgen initially with reductions in RNase L ratio and an increase in Karnofsky score. However, six months after cessation of therapy, the patient suffered a relapse as indicated by a decrease in Karnofsky score and a significant increase in the RNase L ratio. Interestingly, for this patient, the RNase L ratio after the cessation of therapy and subsequent relapse was much greater (52.9) than even before beginning therapy (8.7). In both the non-responder and relapsed patient, low levels of caspase-3 (C3) that were found may indicate that a block exists in the apoptotic pathway.

Dr. DeMeirleir and colleagues, in this patent, concluded that (A) Increases or decreases in the relative amounts (i.e., ratios) of native RNase L when measured against RNase L-related fragments correlates strongly with the presence or absence of CFS disease, respectively; (B) Increases in apoptosis in PBMCs from CFS patients can be measured by analyzing caspase levels; (C) Increases in apoptosis in PBMCs from CFS patients can be measured by analyzing the relative amount of native RNase L and related fragments; (D) As the ratio of RNase L-related fragments to the remaining native RNase L protein increases above a certain level, the process of apoptosis, as measured by caspase levels, appears to stop then decline even further to sub-normal levels; (E) RNase L-related fragments are likely to inhibit the apoptotic process based on the amino acid sequence comparison of RNase L-related fragments to known inducers of cell activation and growth; (F) Upon successful therapy with mismatched double stranded RNA (i.e. amplitgen), RNase L ratios return to 'normal' or healthy control levels; (G) Upon successful therapy with mismatched double stranded RNA, caspase levels return to 'normal' or healthy control levels; (H) Mismatched double-stranded RNA induces the synthesis of 2'-5'A. In turn, 2'-5'A binds to and activates native RNase L homodimers that in turn induce apoptosis, removing the anti-apoptotic block, allowing for return of normal cellular functions.

However, Dr. DeMeirleir and colleagues have provided exciting information regarding a potential treatment therapy identified in their newest patent. The patent, titled "Methods of treatment of chronic immune diseases," was just issued on July 31, 2003 [25]. ***In this patent, the inventors detailed their findings regarding treatment for multiple sclerosis and chronic fatigue syndrome, both of which the inventors consider to be chronic immune diseases. Treatment includes administering to a human an effective amount of a protease inhibitor where this protease inhibitor is an elastase inhibitor and where the elastase inhibitor is a beta-lactam containing compound such as the cephem cefoperazone.*** Furthermore, the inventors state that the diagnosis of chronic immune disease is accomplished by detecting the presence of RNase L fragments. This comes as no surprise because of the volumes of previous work on RNase L as well as the inventors previous patents. In fact, another research group has recently published that RNase L ratios could distinguish CFS patients from healthy controls [26].

Briefly stated, Dr. DeMeirleir and colleagues demonstrated that the enzyme elastase is able to generate fragments of recombinant RNase L protein, the size of which approximates the fragment of native RNase L protein found in peripheral blood mononuclear cells from patients with MS and CFS. This is in-line with a previous publication by DeMeirleir and others [27] where RNase L proteolysis could be mimicked by combining recombinant RNase L with human leukocyte elastase. In the patent, the inventors were able to demonstrate that pure / recombinant RNase L (> 95%) was proteolytically cleaved by elastase and the fragments of RNase L generated by elastase digestion were compared to the size of the fragments of native RNase L found in PBMCs from CFS patients. The inventors demonstrated that the pure RNase L cleavage product (37 kDa) was equivalent in size to that found in CFS patients. ***The inventors then tested the effects of the beta-lactam based antibiotic cefoperazone (Pfizer Pharmaceuticals, trade name Cefobid) on the levels of RNase L protein in the human monocytic leukemia cell line U937. The inventors demonstrate that cefoperazone is able to inhibit the production of the low-molecular weight fragment of native RNase L protein and that this inhibition is dose dependent. They also demonstrate that cefoperazone is able to inhibit elastase***

activity as indicated by a reduction of low-molecular weight RNase L protein by in-vitro testing. This indicates that beta-lactam based antibiotics have an effect on elastase, via its inhibition, and thereby are able to reduce fragmentation associated with RNase L cleavage. In fact, from the previous publication, **the authors state that enhanced human leukocyte elastase activity appears to be involved in the increased proteolysis of RNase L in CFS PBMCs.** Since the inventors listed numerous antibiotics that contain beta-lactam compounds, without discussing their targeted sensitivity, this could help explain why some patients are at least partially responsive to certain antibiotics and why they have reported that they feel better on antibiotic therapy. If their antibiotics contain beta-lactam based compounds then elastase would be inhibited and this would potentially move the patient in the direction of native RNase L restoration by reducing the cleavage products!

A brief look by the NCF into the role(s) of human leukocyte elastase provided us with answers to many additional questions that we had. One of these was the fact that elastase has been found in significant amounts in several different types of amyloidoses [28] and in amyloid fibrils themselves [29,30]. The NCF had noted previous research that examined the molecular basis of CFS [31]. In this work, a unique urinary marker contained N-methylpyrrolidine and was found to be highly significant in CFS patients. However, N-methylpyrrolidine has already been found to accelerate the aggregation process associated with amyloid formation [32]. These findings strengthen our NCF hypothesis for potential amyloid involvement and protein misfolding in the pathogenesis of CFS.

Elastase has been found to cleave all six insulin-like growth factor binding proteins (IGFBPs) in-vitro and in-vivo with a significant proteolytic cleavage of IGFBP-3 [33]. IGFBP's are associated with the proliferative effects of insulin-like growth factors (IGFs) on various cells. Interestingly, IGFBP-3 has been found to upregulate STAT1 and to increase its protein expression [34]. Acclidine therapy, as suggested by Dr. DeMeirleir, acts by increasing IGFBP-3 [19]. Thus, by increasing the levels of IGFBP-3 in the cell, IGF-1 is blocked from binding to its receptor thereby suppressing the growth of the cell, promoting apoptosis, and counteracting the loss of functional p53 protein on the growth of the cell. Additionally, lycopene, which is a natural carotenoid found in tomatoes and readily available as a supplement, has also been found to increase IGFBP-3 as well [35,36].

Elastase has been implicated in chronic inflammation [37] including rheumatoid arthritis [38] and is the target of antirheumatic drugs [39]. In fact, structural changes have been observed in the skin [40] by elastase and in the kidney [41] elastase mediates glomerular injury in-vivo causing proteinuria due to changes in glomerular permeability. Elastase has also been found to cleave the T4-binding globulin (TBG) [42]. TBG serves to maintain an important serum pool of thyroid hormones and to prevent their excessive loss in urine. Elastase also cleaves the corticosteroid-binding globulin reducing its hormone-binding affinity and capacity [42]. In addition, elastase is involved in fibrinolysis [43] as it has been found to degrade fibrin and inhibit the blood coagulation system by degrading key proteins.

However, other interesting findings for elastase are that it regulates Stromal cell-derived factor-1 (SDF-1)/CXCR4 binding [44]. This is particularly intriguing because Human Herpes Virus-6 (HHV-6) uses the CXCR4 receptor for infection [45]. HHV-6 has been implicated in the pathology for both multiple sclerosis and chronic fatigue syndrome [46]. Furthermore, elastase has been found to cleave CD4 (helper cells) and CD8 (cytotoxic/suppressor cells) lymphocytes leading to a reduction in number of these cell types [47]. In addition, elastase has also been found to cleave immune complexes and to regulate inflammation by a feedback mechanism that can lead to cyclic inflammatory states [48].

The finding that Dr. DeMeirleir and colleagues found beta-lactam antibiotics to be associated with the inhibition of human leukocyte elastase is echoed by various publications [49]. However, the NCF has identified one sensitive inhibitor of leukocyte elastase that is readily available and it is boswellic acid [50].

Dr. DeMeirleir's colleagues also have a recent patent application for the cleavage of actin [51]. In this application, the inventors found correlations between the relative amount of native RNase L protein in PBMC extracts and the relative amount of native actin protein in serum. They also found correlations between the ratio of RNase L fragments and the ratio of actin fragments. Actin is a protein that assists in the diverse activities of the cytoskeleton of the cell which includes cell signaling. Interestingly, elastase has been shown to cleave actin in Behcet's disease, a disorder that involves inflammation of the blood vessels [52].

In conclusion, it is obvious that Dr. DeMeirleir and his colleagues, in cooperation with other medical researchers, have investigated several key mechanisms in the pathogenesis of CFS and MS and these included STAT1, RNase L cleavage, actin, and the use of elastase inhibitors as a potential therapy for these chronic diseases. Hopefully by highlighting this research and explaining how it fits within a larger disease framework, the NCF has acted in a responsible manner to inform, educate, and to provide much needed hope to patients, their physicians, and to researchers worldwide.

Part two article review at a glance:

Dr. DeMeirleir and colleagues scientific finding:

- * Expanded model for RNase L fragmentation identified (three fragments) in CFS patients
 - * Fragment #1 is identified with NF-kappaB mimicry
 - * Fragment #2 is identified with 2'-5'A binding
 - * Fragment #3 is identified with Cdk6 chain A mimicry
- * Apoptosis (cell death) increases initially in CFS and then is inhibited when the levels of RNase L related fragments reach a certain point.

Scientific interpretation:

- * Expanded RNase L model helps to explain immune system dysfunction in CFS.
- * Use of leukocyte elastase inhibitors may potentially treat patients with CFS as well as MS due to its impact on RNase L and STAT1.
- * Beta-lactam based antibiotics act as leukocyte elastase inhibitors, however drug sensitivity is most likely unknown for this target.

The National CFIDS Foundation, Inc. provides informative and up-to-date quality scientific research assessments for the CFS patient community, treating physicians, and medical researchers alike. By utilizing evidence-based medical techniques which incorporate the judicious use of intellectual property rights in conjunction with traditional disease profiling methods, investigational reports are generated with the explicit intent of validating the clinical importance and applicability of a discovery as well as to assist in propelling appropriate CFS medical research forward.

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References:

1. *Chronic Fatigue Syndrome: A Biological Approach*; Englebienne P, DeMeirleir K; CRC Press, 2002
2. *The 2-5A Pathway and Signal Transduction: A Possible Link to Immune Dysregulation and Fatigue*; Englebienne P, Herst CV, Fremont M, Verbinnen T, Verhas M, DeMeirleir K; 5: 99-130; in *Chronic Fatigue Syndrome: A Biological Approach*
3. *STATs: Transcriptional control and biological impact*; Levy DE, Darnell JE; Nature Reviews: Mol Cell Bio 2002; 3: 651-662
4. *Impaired response to interferon-alpha/beta and lethal viral disease in human STAT1 deficiency*; Dupuis S, Jouanguy E, Al-Hajjar S, Fieschi C, Al-Mohsen IZ, Al-Jumaah S, Yang K, Chapgier A, Eidenschenk C, Eid P, Al Ghonaium A, Tufenkeji H, Frayha H, Al-Gazlan S, Al-Rayes H, Schreiber RD, Gresser I, Casanova JL; Nat Genet 2003; 33(3): 388-391
5. *STAT1 deficiency unexpectedly and markedly exacerbates the pathophysiological actions of IFN-alpha in the central nervous system*; Wang J, Schreiber RD, Campbell IL; Proc Natl Acad Sci 2002; 99(25): 16209-16214
6. *Targeted disruption of the mouse STAT1 gene results in compromised innate immunity to viral disease*; Durbin JE, Hackenmiller R, Simon MC, Levy DE; Cell 1996; 84(3): 443-450
7. *Targeted disruption of the STAT1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway*; Meraz MA, White JM, Sheehan KC, Bach EA, Rodig SJ, Dighe AS, Kaplan DH, Riley JK, Greenlund AC, Campbell D, Carver-Moore K, DuBois RN, Clark R, Aguet M, Schreiber RD; Cell 1996; 84(3): 431-442
8. *The antitumor effects of IFN-alpha are abrogated in a STAT1-deficient mouse*; Lesinski GB, Anghelina M, Zimmerer J, Bakalakos T, Badgwell B, Parihar R, Hu Y, Becknell B, Abood G, Chaudhury AR, Magro C, Durbin J, Carson III WE; J Clin Invest 2003; 112(2): 170-180
9. US Patent # 6,080,554; issued June 27, 2000; filed April 27, 1999; titled *Methods and compositions for use in characterizing multiple sclerosis disease activity in a subject*; Inventors: Campine IWL, DeMeirleir KL, Herst CVT; Assignee: R.E.D. Laboratories, NV.
10. US Patent # 6,214,554; issued April 10, 2001; filed April 21, 1999; titled *Chronic fatigue syndrome diagnosis*; Inventor: Suhadolnik RJ; Assignee: Temple University of the Commonwealth System of Higher Education
11. *Low expression of interferon-stimulated genes in active multiple sclerosis is linked to subnormal phosphorylation of STAT1*; Feng X, Petraglia AL, Chen M, Byskosh PV, Boos MD, Reder AT; J Neuroimmunol 2002 Aug; 129(1-2): 205-215
12. US Patent Application # 20030017493; published January 23, 2003; filed June 17, 2002; titled *Methods for the detection and treatment of chronic immune diseases*; Inventors: Fremont M, Englebienne P, Herst CVT
13. US Patent Application # 20030077674; published April 24, 2003; filed June 17, 2002; titled *Methods for diagnosis and treatment of chronic immune diseases*; Inventors: Fremont M, Englebienne P, Herst CVT
14. R.E.D. Laboratories website; www.redlabs.com/en/aboutredlabo/redlabohistory.html; website last updated: 1/16/01
15. Flemish Parliament of Belgium; dated June 6, 2001; Flemish Parliament Social Policy Note: Environment and Health, Hearings and Advice, Session 2000-2001, Article 740 (2000-2001); *Testimony of Professor Kenny DeMeirleir*, Human Physiology, VUB hearing of March 5, 2001

16. *Prevention of beta-amyloid neurotoxicity by blockade of the ubiquitin-proteasome proteolytic pathway*; Favit A, Grimaldi M, Alkon DL; J Neurochem 2000; 75(3): 1258-1263.
17. *The protease inhibitor, MG132, blocks maturation of the amyloid precursor protein swedish mutant preventing cleavage by beta-secretase*; Steinhilb ML, Turner RS, Gaut JR; J Bio Chem 2001 Feb 9; 276(6): 4476-4484
18. *Utility of the blood for gene expression profiling and biomarker discovery in chronic fatigue syndrome*; Vernon SD, Unger ER, Dimulescu IM, Rajeevan M, Reeves WC; Dis Markers 2002 18:193-199
19. US Patent Application # 20030017492; published January 23, 2003; filed June 17, 2002; titled *Methods for diagnosis and treatment of chronic immune diseases*; Inventors: Fremont M, Englebienne P, Herst CVT
20. *Immune Cell Apoptosis and Chronic Fatigue Syndrome*; Fremont M, D'Haese A, Roelens S, DeSmet K, Herst CV, Englebienne P; 6: 131-174; in *Chronic Fatigue Syndrome: A Biological Approach*
21. *The STAT family in cytokine signaling*; Ihle JN; Current Opinion in Cell Bio 2001; 13: 211-217
22. *The antitumor effects of IFN-alpha are abrogated in a STAT1-deficient mouse*; Lesinski GB, Anghelina M, Zimmerer J, Bakalakos T, Badgwell B, Parihar R, Hu Y, Becknell B, Abood G, Chaudhury AR, Magro C, Durbin J, Carson III WE; J Clin Invest 2003 July, 112(2): 170-180
23. World Patent # WO0215929; issued February 28, 2002; filed August 16, 2001; titled *Methods and compositions for use in the diagnosis and treatment of chronic immune disease*; Inventors: Englebienne P, DeMeirleir KL, Herst CVT; Applicant: R.E.D. Laboratories, N.V.
24. *Cell death program*; Debatin K; in *Textbook of Malignant Haematology*; Degos L, Linch DC, Lowenberg B, eds. London UK: Martin Dunitz Ltd; 1999: 153-164
25. World Patent # WO03061605; issued July 31, 2003; filed January 10, 2003; titled *Methods of treatment of chronic immune disease*; Inventors: El Bakkouri K, Englebienne P, DeMeirleir K, Herst CVT; Applicant: R.E.D. Laboratories, N.V.
26. *RNase L levels in peripheral blood mononuclear cells: 37-Kilodalton/83-Kilodalton isoform ratio is a potential test for chronic fatigue syndrome*; Tiev KP, Demette E, Ercolano P, Bastide L, Lebleu B, Cabane J; Clin Diag Lab Imm 2003; 10(2): 315-316.
27. *Ribonuclease L proteolysis in peripheral blood mononuclear cells of chronic fatigue syndrome patients*; Demette E, Bastide L, D'Haese A, DeSmet K, DeMeirleir K, Tiev KP, Englebienne P, Lebleu B; J Biol Chem 2002; 277(38): 35746-35751.
28. *Neutrophil proteases associated with amyloid fibrils*; Stone PJ, Campistol JM, Abraham CR, Rodgers O, Shirahama T, Skinner M; Biochem Biophys Res Commun 1993; 197(1): 130-136
29. *The association of an elastase with amyloid fibrils*; Skinner M, Stone P, Shirahama T, Connors LH, Calore J, Cohen AS; Proc Soc Exp Biol Med 1986; 181(2): 211-214
30. *Elastase-type proteases on the surface of human blood monocytes: possible role in amyloid formation*; Lavie G, Zucker-Franklin D, Franklin EC, J Immunol 1980; 125(1): 175-180
31. *Preliminary determination of a molecular basis of chronic fatigue syndrome*; McGregor NR, Dunstan RH, Zerbes M, Butt HL, Roberts TK, Klineberg JJ; Biochem Mol Med 1996; 57(2): 73-80
32. *Nicotine inhibits amyloid formation by the beta-peptide*; Salomon AR, Marcinowski KJ, Friedland RP, Zagorski MG; Biochemistry 1996; 35(42): 13568-13578

33. *Inflammation-related neutral proteases, cathepsin G and elastase, function as insulin-like growth factor binding protein proteases*; Gibson TL, Cohen P; Growth Horm IGF Res 1999; 9(4): 241-253
34. *Identification of STAT-1 as a molecular target of IGFBP-3 in the process of chondrogenesis*; Spagnoli A, Torello M, Nagalla SR, Horton WA, Pattee P, Hwa V, Chiarelli F, Roberts DT Jr, Rosenfeld RG; J Biol Chem 2002; 277(21): 18860-18867
35. *Lycopene supplementation inhibits lung squamous metaplasia and induces apoptosis via up-regulating insulin-like growth factor-binding protein 3 in cigarette smoke-exposed ferrets*; Liu C, Lian F, Smith DE, Russell RM, Wang XD; Cancer Res 2003; 63(12): 3138-3144
36. *Are dietary influences on the risk of prostate cancer mediated through the insulin-like growth factor system?*; Mucci LA, Tamimi R, Lagiou P, Trichopoulou A, Benetou V, Spanos E, Trichopoulos D; BJU Int 2001; 87(9): 814-820
37. *The role of neutrophil elastase in chronic inflammation*; Doring G; Am J Respir Crit Care Med 1994; 150(6 Pt 2): S114-S117
38. *Cathepsin G and elastase in synovial fluid and peripheral blood in reactive and rheumatoid arthritis*; Nordstrom D, Lindy O, Kontinen YT, Lauhio A, Sorsa T, Friman C, Pettersson T, Santavirta S; Clin Rheumatol 1996; 15(1): 35-41
39. *The inhibitory effects of antirheumatic drugs on the activity of human leukocyte elastase and cathepsin G*; Steinmeyer J, Kalbhen DA; Inflamm Res 1996; 45(7): 324-329
40. *Structural changes of human epidermis induced by human leukocyte-derived proteases*; Ludolph-Hauser D, Schubert C, Wiedow O; Exp Dermatol 1999; 8(1): 46-52
41. *The human neutrophil serine proteinases, elastase and cathepsin G, can mediate glomerular injury in vivo*; Johnson RJ, Couser WG, Alpers CE, Vissers M, Schulze M, Klebanoff SJ; J Exp Med 1988; 168(3): 1169-1174
42. *Characterization of T(4)-binding globulin cleaved by human leukocyte elastase*; Janssen OE, Golcher HM, Grasberger H, Saller B, Mann K, Refetoff S; J Clin Endocrinol Metab 2002; 87(3): 1217-1222
43. *The elastase-mediated pathway of fibrinolysis*; Machovich R, Owen WG; Blood Coagul Fibrinolysis 1990; 1(1): 79-90
44. *Leukocyte elastase negatively regulates Stromal cell-derived factor-1 (SDF-1)/CXCR4 binding and functions by amino-terminal processing of SDF-1 and CXCR4*; Valenzuela-Fernandez A, Planchenault T, Baleux F, Staropoli I, Le-Barillec K, Leduc D, Delaunay T, Lazarini F, Virelizier JL, Chignard M, Pidarid D, Arenzana-Seisdedos F; J Biol Chem 2002; 277(18): 15677-15689
45. *Transcriptional down-regulation of CXC chemokine receptor 4 induced by impaired association of transcription regulator YY1 with c-Myc in human herpesvirus 6 infected cells*; Hasegawa A, Yasukawa M, Saksi I, Fujita S; J Immunol 2001; 166(2): 1125-1131
46. *Frequent HHV-6 reactivation in multiple sclerosis (MS) and chronic fatigue syndrome (CFS) patients*; Ablashi DV, Eastman HB, Owen CB, Roman MM, Friedman J, Zabriskie JB, Peterson DL, Pearson GR, Whitman JE; J Clin Virol 2000; 16(3): 179-191
47. *Cleavage of lymphocyte surface antigens CD2, CD4, and CD8 by polymorphonuclear leukocyte elastase and cathepsin G in patients with cystic fibrosis*; Doring G, Frank F, Boudier C, Herbert S, Fleischer B, Bellon G; J Immunol 1995; 154(9): 4842-4850
48. *Elastase from polymorphonuclear leukocytes: a regulatory enzyme in immune complex disease*; Doring G, Goldstein W, Botzenhart K, Kharazmi A, Schiotz PO, Hoiby N, Dasgupta M; Clin Exp Immunol 1986; 64(3): 597-605
49. *Mechanism of inhibition of human leucocyte elastase by monocyclic beta-lactams*; Chabin R, Green BG, Gale P, Maycock AL, Weston H, Dorn CP, Finke PE, Hagmann WK, Hale JJ, MacCoss M et. al.; BioChemistry 1993; 32(34): 8970-8980

50. *Inhibition by boswellic acids of human leukocyte elastase*; Safayhi H, Rall B, Sailer ER, Ammon HP; J Pharmacol Exp Ther 1997; 281(1): 460-463
51. US Patent Application # 20030152919; published August 14, 2003; filed May 15, 2000; titled *Method and compositions for use in diagnosing and characterizing chronic immune disease*; Inventors: Roelens SAM, Englebienne P, D'Haese AMYR, Herst CVT
52. *Characterization of a protease responsible for truncated actin increase in neutrophils of patients with Behcet's disease*; Yamashita S, Suzuki A, Yanagita T, Hirohata S, Toyoshima S; Biol Pharm Bull 2001; 24(2): 119-122