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*Review*

# Impairments of the 2-5A Synthetase/RNase L Pathway in Chronic Fatigue Syndrome

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**Abstract.** *This paper provides an overview of the evidence addressing the impairments of the 2'-5' oligoadenylate (2-5 A) synthetase/RNase L pathway in Chronic Fatigue Syndrome (CFS) patients. The 2-5A synthetase/RNase L pathway in CFS patients appears to be both up-regulated (i.e. increased levels of bioactive 2-5A synthetase and increased activity of the RNase L enzyme) and deregulated (elastase and calpain initiate 83 kDa RNase L proteolysis, generating two major fragments with molecular masses of 37 and 30 kDa, respectively). The deregulation of the 2-5A synthetase/RNase L pathway in CFS accompanies decreased NK-function and deregulation of apoptotic pathways. Since various components of the pathway appear to be related to performance during a graded exercise stress test, some evidence supportive of the clinical importance of the impaired pathway in CFS patients has been provided. Studies addressing the treatment of the deregulation of the 2-5A synthetase/RNase L pathway in CFS are warranted.*

Chronic Fatigue Syndrome (CFS) is known to be a debilitating and complex disorder, characterized by extreme fatigue, which is not improved by bed rest and which may be aggravated by physical or mental activity (1, 2). Apart from fatigue, CFS is accompanied by a wide variety of symptoms, including musculoskeletal pain, neurocognitive impairments, low-grade fever, sore throat, tender lymph nodes, sleep disturbances, headache, *etc.* (1, 2). Although CFS occurs in men and women of all age groups, the typical patient is a middle-class Caucasian woman in her thirties. The majority of the CFS population appears to be within the age range 18-50 years (3, 4). The core feature of CFS diagnosis is the exclusion of any active medical condition which may explain the presence of the symptoms (*e.g.* hypothyroidism, Hepatitis B or C, major depressive disorders with psychotic or melancholic features, bipolar affective disorders, schizophrenia, dementia, alcohol abuse, severe obesity, *etc.*) (1, 2). The impact of CFS on daily functioning is dramatic, with severe activity limitations and participation restrictions (5), including an unemployement rate of 42% of CFS patients (n=2,652) (6). Although there is considerable evidence of an underlying biological process in most CFS patients (7), the pathogenesis of CFS has not been completely delineated. Evidence addressing immune impairments in CFS is accumulating.

Binding of type I interferons (IFN-alpha and -beta), produced in response to infection by a virus or bacteria, to cell membrane receptors triggers an intracellular cascade of events. The expression of 2'-5' oligoadenylate (2-5 A) synthetase genes is increased, generating an accelerated convertion of ATP to 2-5A oligonucleotides that bind to and activate latent ribonuclease (RNase L) (Figure 1). RNase L is one of the intracellular proteins activated by type I interferons and cardinal to the cellular defense mechanism (8). The R462Q variant of RNase L, having about 3-fold reduced catalytic activity *in vitro*, is the most prevalent genetic marker for prostate cancer (9). In relation to the dysfunctional immune system in CFS patients, a number of studies have addressed the 2-5A synthetase/RNase L antiviral pathway in peripheral mononuclear blood cells (PBMC's). Ever since the first report by Suhadolnik and colleagues was published in 1994 (10), the impairments of the 2-5A synthetase/RNase L pathway in CFS patients have been unravelled by scientists around the world. From these studies, it appears that the 2-5A synthetase/RNase L pathway in CFS patients is both up-regulated (*i.e.* increased levels of bioactive

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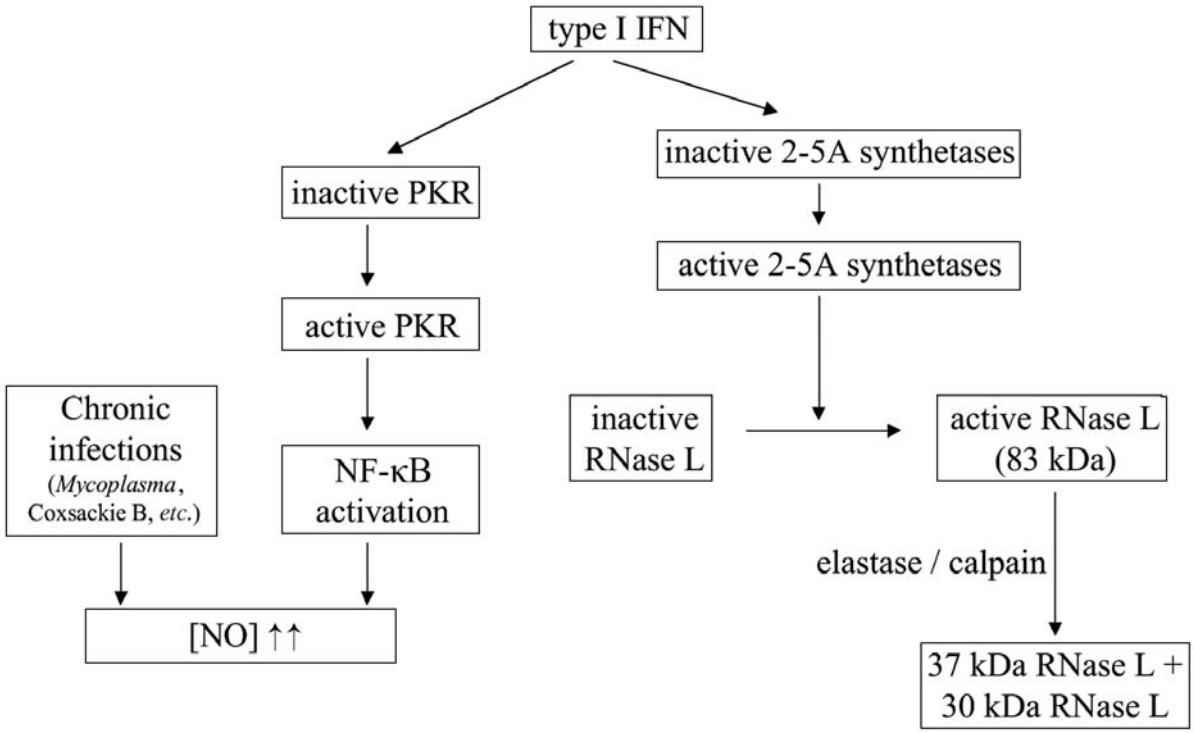


Figure 1. *Intracellular immunopathology in CFS.*

2-5A synthetase and increased activity of the end-product of the pathway, namely the RNase L enzyme) and deregulated (elastase and calpain initiate high molecular weight or 83 kDa RNase L proteolysis, generating two major fragments with molecular masses of 37 and 30 kDa, respectively – Figure 1). The purpose of this paper is to provide the reader with an overview of the published studies which address the 2-5A synthetase/RNase L pathway in CFS patients. First, the evidence regarding the up-regulation of the pathway in CFS patients is discussed, followed by a review of the available data on deregulation of the 2-5A synthetase/RNase L pathway and interactions between the various components of the pathway in CFS patients. Next, the interactions between intracellular infections and the impairmants of the 2-5A synthetase/RNase L pathway are discussed, providing an overall picture of the position of the impaired pathway in the pathophysiology of CFS. The clinical importance of the impairments of the 2-5A synthetase/RNase L pathway in CFS patients is outlined, with special emphasis on associations with disease severity (symptom severity, daily functioning and exercise performance). The limited evidence on treatment of the impairments of the 2-5A synthetase/RNase L pathway in CFS patients is presented. Finally, directions for the further study of intracellular immunopathology in CFS are provided.

## Up-regulation of the 2-5A Synthetase/RNase L Pathway in CFS

In order to assess the 2-5A synthetase/RNase L pathway, a number of key components of the pathway have been monitored (10). Compared to age-, gender- and race-matched healthy controls (n=50), the activity levels of latent 2-5A synthetases, expressed as picomoles of ATP converted to 2-5A per milligram of protein per hour, were lower in CFS patients (n=15) (10). An increased level of bioactive 2-5A synthetase may account for the decreased level of latent 2-5A synthetase, as evidenced by the increased concentrations (the increase over control value ranged from 3.3- to 220-fold) of bioactive 2-5A synthetase seen in all 15 patients with CFS (10). In addition, RNase L activity in PBMC's of all 15 subjects with CFS was markedly elevated over control values (3- to 45-fold elevation over control values) (10). These results were confirmed by three additional studies by the same group, who found significantly elevated bioactive 2-5A synthetase levels and RNase L activity in CFS patients compared to healthy controls (11-13). The level of bioactive 2-5A synthetase was correlated significantly with RNase L activity in two different studies (Spearman's rho=0.54; n=75; $p<0.0001$)

(11) (Pearson's r=0.55; n=53; $p<0.001$) (13), indicating that both aspects of up-regulation of the antiviral pathway accompany one another. The high activity levels of 2-5A synthetase were confirmed in Japanese CFS patients: the mean 2-5A synthetase activity level was 2.5- to 3.0-fold higher in CFS patients (n=44) than in healthy controls (n=38) ($p<0.01$) (14). The 2-5A synthetase activity was judged positive when the percentages of ATP incorporation were over 1.00 (14). Using this approach, 26 out of 44 CFS patients (59%) and 4 out of 38 healthy controls (10.5%) presented with a positive 2-5A synthetase activity test (14). In addition, the down-regulation of the RNase L inhibitor (RLI) may account for the up-regulation of the 2.5A synthetase/RNase L pathway in CFS patients: research revealed a statistically significant decrease in RNase L inhibitor (RLI) mRNA in PBMC's of CFS patients (n=25) compared to healthy controls (n=15) ($p<0.0001$) (15).

Both IFN-alpha and -beta trigger an intracellular cascade of events resulting in increased activity of 2-5A synthetase. However, a relationship between the presence of IFN-alpha in blood plasma of CFS patients (n=40) and 2-5A synthetase activity was found (14). The consistent finding of up-regulation of the 2-5A synthetase/RNase L pathway in CFS patients has been challanged by others who studied gene expression (mRNA expression) of 2-5A synthetase, RNase L, RLI and Protein Kinase R (PKR) in PBMC's of 22 CFS patients, 21 healthy controls and 10 patients with severe gastroenteritis (16). No differences were observed between the CFS patients and healthy controls, but the group with gastroenteritis displayed a marked increase in gene expression for PKR and RLI compared to controls (16). In comparison with the CFS patients, the subjects with gastroenteritis differed with respect to RLI gene expression, which was higher in the latter group (16). The authors correctly indicated that since the 2-5A synthetase/RNase L pathway is routinely activated during infection, the inclusion of patients with infection is required to study the pathway (16). However, they did not study the deregulation of the pathway (see next subheading).

Regarding the stability of the up-regulation of the 2-5A synthetase/RNase L pathway, it was found that during a 24-week clinical trial, RNase L activity increased in 60% of the placebo-treated CFS patients (n=47) (11). Using a time interval of 2 weeks to 8 months, the 2-5A synthetase activity in PBMC's of 5 Japanese CFS patients was tested at least twice. In 4 out of 5 patients the 2-5A synthetase remained high (>1.00) or low, but changed from high to low in 1 subject (14).

Apart from the RNase L enzyme, another dsRNA-dependent antiviral enzyme presents with altered activity in patients with CFS. Besides triggering the 2-5A synthetase/RNase L activation, type I interferons induce the expression of PKR. Activation of this enzyme, as typically seen during viral infection or cellular stress, results in a blockade of protein synthesis and consequent cell death (apoptosis). Conflicting data regarding the activity of the PKR enzyme in CFS patients have been reported: one study found down-regulation of the PKR enzyme in 19 CFS patients compared to 25 healthy controls ($p<0.0001$) (11), another reported activation of the PKR enzyme parallel to the 83 kDa RNase L proteolysis in subsets of CFS (17), while others found no difference in gene expression (mRNA expression) of PKR in PBMC's of 22 CFS patients and 21 healthy controls (16). PKR activation leads to phosphorylation of the inhibitor of NF(nuclear factor)-Î B (IkB) and consequent NF-Î B activation, which in turn causes inducible nitric oxide synthetase (iNOS) expression (18). iNOS generates increased production of NO by monocytes / macrophages, explaining oxidative stress in CFS patients (Figure 1). Elevated nitric oxide (NO) levels have been documented in CFS patients (19), and oxidative stress has been found to be associated with symptom expression in CFS patients (20). In an unpublished study of 16 CFS patients, the PKR activity was within the normal range in the majority of the study sample (11/16), and approximately 50% of the subjects presented with normal NO levels in both monocytes and lymphocytes (21). Further study of PKR activity in CFS patients is warranted, but from the available data it is concluded that the activation status of the PKR enzyme is not characteristic of the CFS population. While some CFS patients appear to have either an up- or down-regulated PKR enzyme, other CFS patients present with a normal PKR activity. Prospective study of this intracellular enzyme is required to examine whether these differences represent different stages of the illness.

## Deregulation of the 2-5A Synthetase/RNase L Pathway in CFS

In 1997, a novel low molecular weight RNase L was discovered in PBMC's from patients with CFS (12). In the absence of the native 83 kDa RNase L, a novel isoform with an estimated molecular mass of 37 kDa (referred to as the low molecular weight) was observed in the PBMC's of CFS patients (12). At that time, it was unclear whether the 37 kDa RNase L was representive of the CFS population in general, and whether the 37 kDa RNase L was characteristic of a particular stage in the course of the illness or if it fluctuates over time (as is the case with symptom severity in the majority of CFS patients). Another study confirmed the presence of the 83 kDa RNase L isoform in CFS patients: while no difference in mean 83 kDa RNase L values were found, the mean values of low molecular weight RNase L was higher in CFS patients (n=53) compared to healthy controls (n=26) ($p=0.007$) (13). The value of 37 kDa RNase L correlated significantly with the value of 83 kDa RNase L (Pearson's r=-.54; $p<0.0001$), but not with the

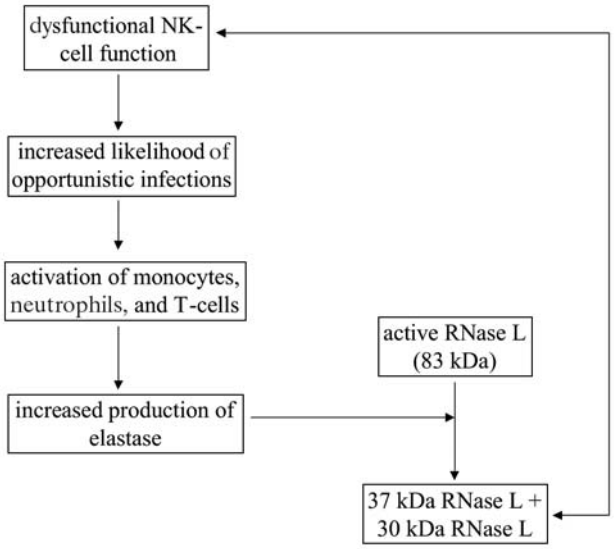


Figure 2. *A hypothetical model for explaining the observed interaction between low natural killer cell function and deregulation of the 2-5A synthetase/RNase L pathway in CFS patients.*

value of IFN-alpha (r=0.13; $p$=0.05) (13). In the same study, all study participants were assessed on three different occasions during a one-year prospective study: the mean 37 kDa RNase L values did not vary significantly from draw to draw in either the CFS patients ($p$=0.49) or controls ($p$=0.35), providing evidence for the stability of the RNase L cleavage over a one-year period (13). One can even speculate that the observed changes in mean 37 kDa RNase L values (4.40) account for the fluctuating nature of CFS symptoms. Studying 57 patients with CFS, 28 healthy controls and 25 subjects with depression or fibromyalgia, it was found that the 37 kDa isoform was more prevalent among CFS patients (50/57) than in healthy volunteers (9/28), depressed subjects (2/14), or patients with fibromyalgia (4/11) (22).

Regarding the etiology of the observed alteration in the 2-5A synthetase / RNase L pathway, it has been shown that the novel 37 kDa RNase L isoform is processed from native RNase L by proteolysis in intact cells (23). Moreover, human leucocyte elastase and calpain, but not caspase-3, have been identified as proteolytic enzymes responsible for the cleaving of 83 kDa RNase L into its truncated form (23, 24). Still, several proteases, known to be present in PBMC's, are able to degrade high molecular weight RNase L and lead to the accumulation of a 37 kDa truncated isoform (23). RLI regulates the activity of the 37 kDa RNase L in PBMC's of CFS patients through protein-protein interaction (25). Both the 37 kDa and the 83 kDa forms of RNase L are structurally similar at the N-terminus, which contains the 2-5A binding domain (25), thus explaining the maintained nuclease activity of RNase L after degradation into its truncated form (23).

The deregulation of the RNase L pathway can be quantified by dividing the amount of 37 kDa RNase L to 83 kDa RNase L, and by multiplying this quotient with 10. It has been suggested that the outcome of this formula, frequently referred to as the RNase L ratio, is normal when it remains below 0.5. In any other case, the RNase L ratio is considered 'increased', suggestive of deregulation of the 2.5A synthetase RNase L pathway. Using the threshold value of 0.5, 41 of the 57 CFS patients (72%) and 3 of the 53 control subjects were found to have evidence of an increased RNase L ratio (22). Using the same approach, Snell *et al.* found an increased RNase L ratio in 46 out of 73 CFS patients (63%) (26), and others reported that no more than 31 out of 186 CFS patients (17%) displayed a 'normal' RNase L ratio (27). Different threshold values for intrepreting the RNase L ratio in CFS patients have been proposed and utilized: using a threshold value of 2.0, 23 out of 27 CFS patients (85%) displayed an increased RNase L ratio (28). From a small study, it was concluded that the cut-off of 0.4 for the RNase L ratio (37 kDa/83 kDa) generated the highest sensitivity and specificity for the diagnosis of CFS (29). Ten out of 11 CFS patients presented with an increased RNase L ratio, compared to 4 out of 14 healthy controls (29). Thus, the sensitivity and specificity of an RNase L ratio of 0.4 for the diagnosis of CFS were 91% and 71%, respectively (29). A higher threshold value is likely to increase the sensitivity, but simultanously decrease the specificity of the RNase L ratio as a biological marker for CFS. A larger study is required to either refute, or confirm the validity of 0.4 as the threshold value for interpreting the RNase L ratio.

The deregulation of the 2-5A synthetase/RNase L pathway appears to accompany different aspects of immune dysfunction in CFS patients. A reduced number and activity (cytotoxicity) of Natural Killer cells (NK-cells) have been reported in patients with CFS (30-32). Comparing 27 CFS patients with 20 healthy volunteers, the number of NK-cells was one of the primary discriminatory functions between the two groups (28). In addition, a negative correlation between the RNase L ratio and both the number and % of NK-cells was observed in CFS patients (r=–0.62 and –0.51 respectively) (28). Thus, the more severe the deregulation of the 2-5A synthetase/RNase L pathway in CFS patients, the lower the number and % of NK-cells. Given the cross-sectional nature of the study, one can only speculate in terms of the chicken and the egg. The NK-cells serve as one of the primary defense mechanisms in the human body. A lower number of NK-cells increases the likelihood of (opportunistic) infections like *Mycoplasma spp*., which in turn activate monocytes, neutrophils and T-cells (Figure 2) (33, 34). To bring about their phagocytic activity, monocytes, neutrophils and activated T-cells produce elastase, which enables them to pass

through connective tissues (35, 36). Research revealed that the proteolytic enzyme elastase is, at least in part, responsible for the proteolysis of the native 2-5A-dependent RNase L into its truncated form (the low molecular weight 2-5A RNase L) (23). Thus, we hypothesize that impairments in NK-cell functioning predispose CFS patients to infections like *Mycoplasma spp.*, which in turn deregulate the 2-5A synthetase/RNase L pathway. Still, in absense of well-designed long-term prospective studies of CFS patients, the exact nature of these interactions remain speculative.

Apart from the observed association with a suppressed NK-function, the deregulation of the 2-5A synthetase/RNase L pathway appears to accompany additional immune characteristics in CFS patients. It has been shown that G-actin, a calpain substrate playing a crucial role in antigen presentation and T-cell signaling, is cleaved in the PBMC's of CFS patients, and the presence of actin fragments correlates with the presence of RNase L fragments ($r=0.71$; $p<0.001$; $n=107$) (24). Given the cardinal role of G-actin in the immune functioning of the human body, its cleavege in the PBMC's of CFS patients might account for a variety of immune abnormalities observed in this population (the studies examining immune functioning in CFS have been reviewed in (37)). In addition, in the PBMC's of CFS patients, the extent of RNase L cleavage was related to apoptotic activity, as assessed by the activity of various caspases (38). The activity of both caspase-8 and caspase-3, but not caspase-2 or caspase-9, in PBMC extracts of CFS patients was significantly increased ($p<0.01$) in samples with an RNase L ratio between 2 and 20. In samples characterised by higher ratios, the activity of both enzymes returned to normal. This down-regulation of apoptotic pathways was further evidenced by a significantly decreased activity of caspase-2 and caspase-9 in samples with very high RNase L ratios. Thus, from these data it can be concluded that apoptotic pathways in the PBMC's of patients with CFS are activated in case of an RNase L ratio between 2 and 20, and blocked in patients with a very high RNase L ratio (38). The above outlined G-actin cleavage in the PBMC's of CFS patients is likely to take part in the deregulation of the apoptotic pathways in samples of CFS patients: actin fragmentation plays a critical role in the (activation of) apoptotic processes.

## Associations between Impairments of the RNase L Pathway and Infections in CFS

In a study published eleven years ago, it was shown that all 14 studied CFS patients were positive for human herpes virus type 6 (HHV-6) expression, confirmed by means of monoclonal antibodies to HHV-6 (10). The HHV-6 expression accompanied the up-regulation of the 2-5A synthetase/RNase L pathway, as evidenced by the increased levels of bioactive 2-5A synthetase and the elevated RNase L activity in the PBMC's of the CFS patients (10). The latter data suggested a comorbid pathophysiological pathway between infection with HHV-6 and up-regulation of the 2-5A synthetase/RNase L pathway in CFS patients. Indeed, overstimulation of the cellular defense mechanism due to viral reactivation, as typically seen in HHV-6 infections, might lead to increased levels of bioactive 2-5A synthetase and consequent elevated RNase L activity. In addition, the restoration of the 2-5A synthetase/RNase L pathway due to treatment with poly(I):poly($C_{12}$U) was accompanied by a sustained disappearance of HHV-6 in 77% of the study sample (10).

In a study of 22 CFS patients, a correlation ($p<0.05$) between the activity of 2-5A synthetase and the antibody titer of Epstein Barr Virus, but not to the antibody titers of *Coxiella burnetti*, was found (14). A correlation coefficient was not reported, making it difficult to intrepret the strength of the observed association. In a large study of 186 consecutive CFS patients, the mean logarithmic transformed RNase L ratios were significantly higher in the patients showing evidence of infection with *Mycoplasma spp.* compared to patients without evidence of a current *Mycoplasma* infection ($p=0.016$) (27). Both groups (CFS patients with and without infection by *Mycoplasma spp*.) displayed no difference in any demographic characteristic. The latter observation suggests a comorbid pathophysiological pathway between *Mycoplasma spp*. and deregulation of the 2-5A synthetase/RNase L pathway in patients with CFS. As outlined above, infections like *Mycoplasma spp*. activate monocytes, neutrophils, and T-cells (33,34). To bring about their phagocytic activity, monocytes, neutrophils, and activated T-cells produce elastase, which degrades the native 2-5A dependent RNase L into its truncated form (Figure 2). Thus, on a theoretical basis, *Mycoplasma spp*. are capable of deregulating the 2-5A synthetase/RNase L pathway in patients with CFS. Conversely, severe deregulation of the 2-5A synthetase/RNase L pathway is accompanied by down-regulation of apoptotic activity in PBMC's due to accumulation of proteolytic cleavage products (38). Initial up-regulation of apoptosis in PBMC's of CFS patients due to deregulation of the 2-5A synthetase/RNase L pathway is followed by down-regulation (38). A down-regulated apoptotic activity implicates a suppressed ability to eliminate intracellular antigens like *Mycoplasma spp*.

## Clinical Importance of Impairments of the RNase L Pathway in CFS

In order to be a biological gradient, a correlation between the biological parameter of interest (*i.e.* impairment of the RNase L pathway) and the clinical severity of the disorder of interest (*i.e.* CFS) is required (39). Evidence supporting the

clinical importance of RNase L activity in CFS patients has been provided by the outcome of a randomised, placebo-controlled, clinical trial: the decrease in RNase L activity due to 24 weeks of treatment with poly(I):poly($C_{12}$U) was associated with cognitive improvement in the treatment group (n=45; Spearman's rho=0.60; $p$=0.007), but not in the placebo group (11). In addition, a statistically significant correlation was observed between the Karnofsky Performance Scale and both the level of bioactive 2-5A synthetase/RNase L activity and bioactive 2-5A concentration in 53 CFS patients (r=–0.21; $p$=0.002 and r=–0.15; $p$=0.025, respectively) (13). However, we are unawere of studies addressing the psychometric properties of the Karnofsky Performance Scale (in CFS patients), and the observed correlation coefficients were very low. To interpret a correlation analysis, one should focus on the correlation coefficient rather than interpreting the $p$-value. Correlation coefficients as low as 0.2, regardless of the $p$-value, suggest no association is present. Thus, the above outlined data are not supportive of the clinical importance of the impairments in the 2-5A synthetase/RNase L pathway. Likewise, the observed correlation between RNase L activity and the total score of the Metabolic Screening Questionnaire (r=0.20; $p$=0.01), a questionnaire reflecting general health, is too weak to be supportive of the clinical importance of RNase L activity in CFS patients.

If impairments of the 2-5A synthetase RNase L pathway are of clinical importance to CFS patients, then one would expect an association between these intracellular immune impairments and performance during an exercise stress test. Studying 73 diagnosed CFS patients, it was concluded that the patients with evidence of deregulation of the 2-5A synthetase RNase L pathway (*i.e.* RNase L ratio ≥0.5) had a lower peak oxygen uptake and exercise duration during a maximal, graded exercise test than the CFS patients with a 'normal' RNase L ratio (26). In contrast, the former group displayed a higher mean score on the Karnofsky Performance Scale, suggestive of better daily functioning (26). A step-wise discriminant function analysis with peak oxygen uptake, exercise duration and the Karnofsky Perfomance Scale score as the independent variable, and classification according to the RNase L ratio as the dependent variable, revealed that all three variables entered the single significant function. Peak oxygen uptake contributed most to the difference between the groups, followed by exercise duration and the Karnofsky Perfomance Scale score, respectively (26). These results were compared by a similar study from the same group controlling for potentially confounding gender effects (40). Thus, preliminary evidence for an association between intracellular immune dysfunction and exercise performance in CFS patients was provided. In an unpublished study of 16 CFS patients, strong correlations (r ranged between 0.65 and 0.73) were observed between four intracellular immune parameters (*i.e.* elastase activity, PKR activity, RNase L activity and cleavage) and both the resting respiratory exchange ratio and the oxygen uptake at the anaerobic threshold (defined as a respiratory exchange ratio of 1.0) (21). The achieved workload at the anaerobic threshold correlated with the PKR activity. Forward stepwise multiple regression analysis revealed: i) that elastase activity in PBMC's was the only factor related to the reduction in oxygen uptake at a respiratory exchange ratio of 1.0 (regression model: $R^2$=0.53, F(1,14)=15.5, $p$<0.002; elastase $p$<0.002); ii) that the PKR activity inside PBMC's was the principle factor related to the reduction in workload at a respiratory exchange ratio of 1.0; iii) that elastase activity was the principle factor related to the reduction in % of target heart rate achieved (21). These data add evidence for an interaction between intracellular immune deregulation (*i.e.* elastase activity, RNase L activity and cleavage, and PKR activity in PBMC's) and exercise performance in patients with CFS. To establish a causal relationship, further study of these interactions in a larger study sample and the use of a prospective longitudinal design is required.

In a cross-sectional study of 137 patients with CFS, the subjects underwent pulmonary function testing, histamine bronchoprovocation testing and phlebotomy (immuno-phenotyping and RNase L ratio determination) (41). Bronchial hyperresponsiveness was defined as a decrease of at least 20% in forced expiratory volume in one second compared to baseline values on inhalation of a cumulative dose of histamine of less than, or equal to 20 mg. Comparing patients with (n=73) and without (n=64) evidence of bronchial hyperresponsiveness, no differences in mean RNase L ratio ($p$=0.06) or the number of patients presenting with an RNase L ratio >2 were found (41). These data refute any interaction between RNase L cleavage and bronchial hyperresponsiveness. Nevertheless, bronchial hyperresponsiveness appears to fit our current understanding of CFS pathophysiology: the same study showed that CFS patients with bronchial hyper-responsiveness, compared to CFS patients with normal bronchial responsiveness, have more pronounced immune activation (evidenced by the increased numbers and percentages of cytotoxic T-cells and the accompanying decreased percentages of virgin CD4+ cells) (41).

## Treatment of the Impairments of the RNase L Pathway in CFS

Together with the initial report on up-regulation of the 2-5A synthetase/RNase L pathway, Suhadolnik *et al.* (10) reported that 24 weeks of therapy with poly(I):poly($C_{12}$U), a double-stranded RNA (dsRNA) with broad-spectrum antiviral and immunomodulatory activity previously used for the treatment

of both breast cancer and infection with the human immunodeficiency virus, decreased the level of bioactive 2-5A synthetase towards normal in 13 out of 15 CFS patients studied. In addition, RNase L activity decreased significantly in all 15 subjects. The restoration of the 2-5A synthetase/RNase L pathway was accompanied by a sustained disappearance of HHV-6 in 77% of the study sample (10). Poly(I):poly($C_{12}$U) is a mismatched dsRNA better known as Ampligen (HEM Pharmaceuticals, Rockville, MD, USA). These preliminary data were confirmed by a randomised, double-blind, placebo-controlled, multicenter trial examining the effectiveness of poly(I):poly($C_{12}$U) in CFS patients (11). Patients in the treatment group received 200 mg of poly(I):poly($C_{12}$U) twice weekly for 2 weeks, followed by 400 mg twice weekly for 22 weeks. After 24 weeks, statistically significantly more patients in the treatment group displayed a decrease in RNase L activity ($p=0.01$), while the % change in 2-5A synthetase level or the median % change in 2-5A synthetase level did not reach statistically significance ($p=0.08$ and 0.09, respectively) (11). Still, the decrease in RNase L activity was associated with cognitive improvement in the treatment group (Spearman's rho=0.60; $p=0.007$), and not in the placebo group (11).

We are unaware of other published studies addressing the treatment of the impaired 2-5A synthetase/RNase L pathway in CFS subjects. Studies examining the treatment of the deregulation of the pathway (*i.e.* 83 kDa RNase L cleavage) in patients with CFS are essentially lacking.

## Future Directions: Elastase-triggered Channelopathies in CFS?

The recent data suggesting a cardinal role for the elastase enzyme in intracellular immunopathology in CFS might encourage researchers to examine the consequences of increased intracellular elastase activity in CFS patients. Indeed, elastase degrades elastin, releasing soluble peptides with interesting biological properties (42). As early as 1987 it was discovered that elastin-derived peptides, released due to cleavage of elastin by elastase, markedly stimulate the calcium influx, inhibit the calcium extrusion by a calmodulin-dependent mechanism and stimulate the sodium influx (possibly as a consequence of an inhibited sodium-potassium-ATPase) (42). The authors speculated that the increased calcium influx might trigger activation of monocytes. Thus, increased elastase activity, as seen in the PBMC's of CFS patients, might trigger a channelopathy distinct from, but resembling, the one previously suggested in CFS patients (28, 43-45). As outlined previously (43), a marked calcium influx into the cells requires a large amount of calcium, which can be obtained from dietary and calcified tissue turnover sources. Thus, elastase might trigger osteoclasts to degrade the bone matrix and to release calcium into the blood, leading to depleted bone mineral density or even osteoporosis.

Together with the previously suggested channelopathy in CFS patients (28, 43-45), the elastase-triggered channelopathy might be an issue for future research. Starting from the N-terminal end of the RNase L polypeptide, the first 330 amino acids sequence present a high degree of homology with the ankyrin repeat motif. Proteolytic cleavage of 83 kDa RNase L generates ankyrin repeat motif-containing fragments. The ankyrins are a family of proteins that control numerous physiological processes by means of interactions with integral membrane proteins (46). In particular, ankyrin proteins are capable of associating with ABC transporters which they link to the cytoskeleton (45). RLI impairs 2-5A binding on the ankyrin domain of RNase L (47) and, consequently, RLI binds ankyrin fragments released in the cells of CFS patients. RLI is part of the ATP binding cassette (ABC) transporter superfamily. When the ankyrin fragment of RNase L is released by cleavage, it also competes with the cognate ankyrin protein ABC transporter, inducing deregulation of their proper function. Recent research revealed sequence similarity between RLI and several ABC transporters, for instance the sulphonylurea receptor (SUR 1) (43). SUR 1 is an important member of ATP-sensitive potassium channels. Impairment of SUR 1 function in cells could be postulated to lead to extreme losses of both cellular potassium and magnesium ($K^+$ efflux and interdependent $Mg^{2+}$ flux). This type of channelopathy has been suggested to account for the majority of the signs and symptoms of CFS (43). To date, only one study has aimed at finding evidence supportive of this type of channelopathy in CFS patients (28). If the outlined channelopathy is present in CFS patients, then one would expect to find an association between 83 kDa RNase L cleavage (*i.e.* RNase L ratio) and serum potassium (or even with the ratio of serum potassium over non-serum potassium). This assumption was not supported by the study results, refuting the presence of this type of channelopathy in CFS patients. Further study of different kinds of channelopathies in larger study samples of CFS patients is required.

## Conclusion

A number of studies from independent laboratories have provided evidence for the up-regulation of various components of the 2-5A synthetase/RNase L pathway in patients with CFS. Both the levels of bioactive 2-5A synthetase and RNase L activity are increased in the PBMC's of CFS patients compared to healthy controls. In addition, evidence regarding the deregulation of the 2-5A synthetase/RNase L pathway in CFS patients is accumulating. A novel 37 kDa RNase L isoform has been repeatedly identified in the PBMC's of major subsets of the CFS population. The 37 kDa RNase L is processed from

proteolysis of the native 83 kDa RNase L by human leucocyte elastase and caplain. The deregulation of the 2-5A synthetase/RNase L pathway in CFS accompanies decreased NK-function and deregulation of apoptotic pathways. The latter supports the preliminary evidence suggestive of a comorbid pathophysiological pathway between infections (*e.g. Mycoplasma spp*., HHV-6) and impairment of the 2-5A synthetase/RNase L pathway in CFS. Still, further study of the 2-5A synthetase/RNase L pathway in patients with CFS, using prospective studies including control groups with known infection (*e.g.* gastroenteritis), is required to confirm these observations. Limited evidence regarding the clinical importance of the impairments of the 2-5A synthetase/RNase L pathway in CFS has been provided: various components of the pathway appear to be related to the performance during a graded exercise stress test. On the other hand, bronchial hyperresponsiveness is frequently observed in CFS patients, but appears not to be related to RNase L cleavage. From the available literature, it appears that poly(I):poly($C_{12}$U), a broad-spectrum antiviral and immunomodulating drug, is capable of restoring the elevated RNase L activity towards normal and improving cognitive functioning in CFS patients. No data addressing the efficacy of poly(I):poly($C_{12}$U) in altering the deregulation of the 2-5A synthetase/RNase L pathway in CFS patients are currently available. Further study of the various components of the pathway, their clinical importance, fluctuating nature, exact place into CFS pathophysiology and treatment implications in large samples of CFS patients is warranted.

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