

INVITED REVIEW

RNase L in Health and Disease -- What Did We Learn Recently?

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ABSTRACT. The 2',5'-oligoadenylate-dependent ribonuclease L (RNase L) is central to the innate cellular defense mechanism induced by type I interferons during intracellular infection. The protein, activated by 2',5'-oligoadenylates precludes the replication of the infectious agent by cleaving single-stranded RNA and, along with the double-stranded RNA-dependent protein kinase, its spreading by inducing the cell to undergo suicide (apoptosis). In absence of infection, the protein remains dormant. Recent evidence indicates, however, that the protein is activated in absence of infection and may play a role in cell differentiation, immune activation, and act as a tumor-suppressor. A deregulation in this pathway has been documented in immune cells of chronic fatigue syndrome patients which involves the presence of a catalytically active truncated RNase L. This protein escapes the normal regulation which implies the development of a cascade of unwanted cellular events. The present article reviews our current understanding of this deregulation, enlightens its relevance in the pathological process and proposes new targets for therapeutic development.

KEYWORDS. RNase L, 2',5'-oligoadenylate synthetase, type I interferon, chronic fatigue syndrome, double-stranded RNA-dependent protein kinase

INTRODUCTION

Since the discovery by Suhadolnik and colleagues (1) of an abnormality in the 2',5'-oligoadenylate-dependent ribonuclease (ribonuclease L, RNase L) pathway in chronic fatigue syndrome (CFS), the way has been long to

understand its origin and possible consequences. Our understanding of the situation at cellular and clinical levels has now matured to such a point that a comprehensive review is certainly more than welcome. In fact, the title of this article could have rather read "What do type I interferons do in health and disease?" Indeed, the RNase L story takes part in a complex system of signal transduction mechanisms induced in almost all of our cells by type I interferons (IFN-alpha/beta) (2) of which the various players are differentially regulated by these related cytokines (3). Abnormal activation of the innate immunity IFN-alpha/beta pathway is progressively surfacing in the pathogenesis and maintenance of so far poorly understood clinical conditions, including not only CFS (4), but also lupus (5), rheumatoid arthritis (6) and type I diabetes (7-9). Unfortunately, our current knowledge remains limited and the cause for this sustained activation (or lack of activation) of specific signaling in type I IFN pathways, even in the absence of any infectious agent detectable by our limited means, is speculative and most probably multifactorial, particularly in CFS (10). Therefore, more research efforts will be required before we understand fully the origins of the IFN-alpha/beta pathway deregulations in these disorders. Meanwhile, however, our understanding of the origin and consequences of the RNase L deregulation in CFS offers the opportunity to fully appreciate the value of the truncated form as a biochemical marker (11), the detection of which allows for the implementation and follow-up of new possible therapeutic interventions.

WHY IS RNASE L SO CENTRAL IN THE PATHWAY?

IFN-alpha/beta are normally produced by cells as a response to infection by several pathogens including viruses, bacteria, chlamydia, mycoplasma, or as a response to specific cytokines such as tumor necrosis factor (12). At first, it was thought that IFN-alpha/beta were produced only as a response to the double-stranded (ds)-RNA produced by intracellular infections. Currently however, new evidence shows not only that single-stranded (ss)-RNA can activate the IFN cascade intracellularly (13), but also that Toll-like receptor 3 at the surface of specialized cells is capable of sensing ds-RNA and inducing IFN-alpha/beta production (14). Finally, bacterial DNA is susceptible to endocytosis and activates Toll-like receptor 9 intracellularly (15), which induces the cell to produce IFN-alpha (16). Interestingly, it has been recently noted that DNA sequences capable of inducing cells to produce IFN-alpha could be more frequent in eukaryotic genomes than previously thought, which may have important implications for immune responses and the development of autoimmunity (17).

By binding to cell membrane receptors, IFN-alpha/beta trigger a cascade of cellular events that ends up in the induction and production of proteins that are central to the cellular defense mechanisms. RNase L is just one among the many proteins involved that are listed in Table I. Why then is RNase L so important in bringing up the deregulations observed in CFS and other illnesses? Not just because of the presence of an abnormal form of the protein in some pathological conditions but merely because it stands, along with the ds-RNA-dependent protein kinase (PKR) and the 2',5'-oligoadenylate synthetases (2-5OAS), in the first line of the innate cellular defense mechanism induced by the IFN pathway (Table I). Therefore, understanding the RNase L deregulation allows one to unravel more profound disturbances upstream as well as downstream of the IFN pathway, that can be associated with several pathologies, including CFS. The importance of the protein in maintaining cellular homeostasis is best exemplified by the recent detection of two germline mutations in the RNase L (rnl) gene in familial prostate cancer (18-19). These mutations result in the expression of a truncated, completely inactive protein (18). This further supports a tumor-suppressing role for the protein.

WHAT DOES RNase L DO IN HEALTH?

The RNase L protein is latent in nearly every cell type as a monomeric inactive endonuclease of 83-kDa. A three-dimensional molecular model of RNase L is depicted in Figure I, which features also the domains of the protein. In the N-terminal region, the protein contains nine ankyrin repeats, the two penultimate of which contain the two P-loops which make the 2',5'-oligoadenylates (2-5A)-binding site. The C-terminal region contains the catalytic site. In between is a protein kinase homology domain containing a cysteine-rich region. The cellular response to IFN-alpha/beta induces the production of the latent RNase L along with the 2-5OAS and the 2-5OAS-like proteins recently suggested to be capable of interacting with the thyroid hormone receptor as co-repressors (20).

[Table 1: Non-Exhaustive List of Proteins Induced by Type I Interferons and Their Cellular Function

See <http://www.cfids-cab.org/freds/T-1.jpg>]

[Figure 1: Three dimensional model of human RNase L featuring its characteristic domains. The model is shown in tube representation and the

Gly-Lys-Thr residues of the two P-loops which make the 2-5A-binding site (arrows) are in ball representation.

See <http://www.cfids-cab.org/freds/F-1.jpg>]

[Figure 2. Involvement of RNase L in healthy cell regulation and defense.

See <http://www.cfids-cab.org/freds/F-2.jpg>]

The 2-50AS polymerize ATP into 2-5A which bind to and activate the dormant RNase L. Activation occurs by homodimerization (21) which confers catalytic activity to the enzyme. The homodimerization occurs through the interaction of the kinase homology domain of both monomers (22). For the homodimerization and activation to occur, the 2-5A oligomer must be at least a trimer (21). As schematically summarized in Figure 2, the activated enzyme is capable of degrading RNA by endonucleolysis at specific base dyads and this activity is regulated by a natural RNase L inhibitor (RLI), the expression of which is not under interferon control (23). RLI is a member of the ATP-binding cassette super family of ion channel proteins (24) and is likely to localize at the plasma membrane where it may play other roles, including the recruitment of viral proteins for post-translational modification (25). Up to recently, it was thought that ss-RNA of viral origin and cellular ribosomal RNA (rRNA) were the only targets of RNase L (26). More recent evidence shows, however, that cellular messenger RNA (mRNA) are also specific targets of RNase L (Figure 2).

In specialized cells such as muscle cells, the activated RNase L-RLI system plays a role in cell differentiation by regulating the mRNA of central transcription factors that control the cell maturation (27). The system further controls cell suicide mechanisms (apoptosis) since it has been demonstrated that RNase L targets specific mitochondrial mRNAs (28), the cleavage of which results most probably in a change of mitochondrial membrane potential and permeability permitting the release of cytochrome c (cyt c) which further activates downstream caspases, a process regulated by IFN-beta (29). Most importantly, in immune cells (monocytes and lymphocytes), IFN-alpha/beta induce two sensitive genes called *Isg15* and *Isg43*, of which the mRNA is regulated by RNase L (30). *ISG 15* is on the one hand a small unique 15-kDa protein which functions both within and outside the immune cells (31). Intracellularly, *ISG 15* functions as an ubiquitin homologue which forms conjugates with cellular proteins (32), including a member of the SERPIN (serine protease inhibitor) super family termed *Serpin 2a* (33). The *ISG 15* conjugation targets these proteins for degradation by the proteasome system

(34). However, ISG15 is also secreted by immune cells (31) and acts as a cytokine inducing the production of IFN- γ by T-cells. This concomitantly results in the proliferation of natural killer (NK) cells and the induction of cytolytic activity by NK-derived lymphokine-activated killer (LAK) cells (35). ISG43 is on the other hand a 43-kDa protease that specifically removes ISG 15 from its conjugates (36). The ISG43 mRNA is also susceptible of activating 2-50AS (30). Consequently, the mRNA endonucleolysis by RNase L regulates the ubiquitination of proteins and IFN- γ production by the ISG15-ISG43 interplay (30,37) as well as its own activation (30). Thus, these recent data indicate that the RNase L/RLI system role is not limited to the cell defense mechanism against intracellular infection but extends to the complete innate and adaptive immune systems, including NK and T-cell proliferation and activation, as well as to cell differentiation and proliferation.

THE ORIGIN AND EFFECTS OF RNase L DYSFUNCTION

The preliminary observations of Suhadolnik and colleagues (1) of RNase L abnormalities in CFS were made in peripheral blood mononuclear cells (PBMC) and counter to what occurs in familial prostate cancer (18), the observed RNase L activity was higher than in normal controls. This abnormal hyperactivation of the RNase L system was later associated with the presence in PBMC of abnormal truncated RNase L-related proteins (38). In particular, a truncated protein of 37-kDa was shown to retain the 2-5A-dependent catalytic activity of RNase L, and to remain capable to interact with RLI (11,39-40). It was quite rapidly recognized that these proteins were produced by proteolytic cleavage of the native enzyme rather than by gene splicing, and the proteases involved were identified as human leukocyte elastase and m-calpain (41-42).

The structure of a cleavage product of RNase L containing both the 2-5A-binding site and the catalytic activity (see Figure I) remain presently speculative but the reassociation of N- and C-terminal fragments through a disulfide bridge remains a realistic possibility (40-41). However, besides the 2-5A-binding 37-kDa fragment, a 30-kDa fragment containing only the catalytic site of the enzyme is also generated by proteolytic cleavage, which can be active in a 2-5A-independent manner (41). Interestingly, other data from our group indicate that the presence of RNase L fragments in PBMC is directly related to the preferential production by 2-50AS of 2-5A dimers. Instead of higher 2-5A oligomers, the dimers bind to the monomeric inactive RNase L, but do not induce its homodimerization and activation (21).

This therefore suggests, as schematically depicted in Figure 3, that normal RNase L activation does not occur and that the monomeric RNase L is the substrate for proteolytic degradation. The RNase L deregulation could therefore find its origin either in the abnormal activation of 2-50AS, or in the preferential induction by IFN-alpha/beta of the p100 2-50AS isoenzyme, respectively, to produce preferentially 2-5A dimers. Indeed, counter to the p41 and p69 isoforms of 2-50AS which produce higher 2-5A oligomers, the p100 2-50AS produces preferentially dimers (43).

The presence of unregulated active RNase L fragments in immune cells may lead to deleterious effects which are inherent to the cellular targets of the protein, as further shown in Figure 3. First, an unregulated destruction of rRNA and of mitochondrial mRNA leads to cell apoptosis. This process further increases the activation of pro-apoptotic and pro-inflammatory proteases, including m-calpain and elastase, both of which have been identified as responsible for RNase L cleavage. This process thus accelerates the RNase L deregulation. Second, a targeted attack of ISG15 and ISG43 mRNAs leads to a decreased capacity of the cell to produce the proteins. This results in a reduced rate of ubiquitination and deubiquitination of cellular proteins. This imbalance in the IFN-dependent regulation of the ubiquitination of selected cellular proteins, including the IFN-induced serine protease inhibitor Serpin 2a, therefore has a direct negative impact on intra-cellular elastase (a serine protease) regulation. Third, the disappearance of ISG43 mRNA further deregulates 2-50AS activation. Finally, the incapacity of the activated cells to secrete ISG 15 has two major consequences in the cross-talk between innate and adaptive immunity: a lack of proper stimulation of T-cells for the production of IFN- γ and a reduced proliferation and activation of NK and LAK cells, respectively.

[Figure 3: Generation and damaging effects of the unregulated truncated active RNase L in immune cells.

See <http://www.cfids-cab.org/freds/F-3.jpg>]

Currently, we do not know if the RNase L deregulation observed in PBMC occurs also in other cell types and any conclusion at the present stage would therefore be rather speculative. However, should the deregulation exist in muscle cells where a specific role has been identified for RNase L in cell differentiation (27), it would necessarily restrain normal muscular

development and hence activity. Since muscular weakness is a common feature of CFS (44), it is tempting to attribute, at least in part, its origin to a possible RNase L deregulation in myocytes. In our opinion, the search for the RNase L deregulation in cell types other than PBMC therefore deserves further focus.

Beside the unregulated catalytically active truncated RNase L, we have previously also shown (45) that the proteolytic cleavage of the endonuclease by m-calpain released fragments containing the ankyrin repeats. By analogy with other ankyrin proteins, we have also suggested that these fragments were capable of interacting with ion channels of the ATP-binding cassette super family strongly related to RLI. This abnormal interaction would induce the channelopathy already clinically documented in CFS (46).

WHAT QUESTIONS DO WE NEED TO ADDRESS AND HOW CAN WE MANAGE THE DYSFUNCTION TODAY?

Our current understanding of the RNase L deregulation is likely to indicate that it is a self-sustained accelerating process (see Figure 3). The best way to block this pathological cascade would be to normalize and regulate the 2-50AS activation to produce suitable 2-5A oligomers. In order to address properly this question, we need to understand the origin of the 2-50AS deregulation: is it the consequence of a bad activation of the lower molecular weight isoforms by abnormal nucleotide sequences or does it result from a preferential induction of the p100 protein? Whilst we do not yet have a complete response to that question, several elements allow us to argue in favor of the first possibility. First, preliminary observations from our laboratory are likely to indicate the presence of abnormally high levels of oligonucleotides in the serum of CFS patients when compared to healthy controls. Abnormally high oligonucleotide levels (particularly if they are short and present a suitable structure) (13) are capable of deregulating the 2-50AS. Second, the clinical efficacy of the mismatched RNA drug Ampligen in CFS is accompanied by a regulation of the 2-50AS activation and a decrease in RNase L dysfunction. Third, preliminary but limited attempts at showing a preferential induction of the p100 protein by PCR were unsuccessful.

To the best of our knowledge, the only drug so far developed and capable of regulating the 2-50AS activity is the mismatched ds-RNA Ampligen (47),

which unfortunately also upregulates PKR (48), which is highly undesirable in CFS (49). Thus, other drugs are required that target more specifically and distinctly the 2-5AS and PKR. Meanwhile, however, we have other means at our disposal capable of reducing the RNase L deregulation process. Calcium antagonists, which have already shown efficacy in CFS therapy (47), reduce m-calpain activation. Elastase inhibitors are another class of drugs that show promise in regulating the dysfunction. Currently, we have reproduced the RNase L deregulation in cell culture models which have allowed testing such drug candidates with some success. Thus, our progressive in-depth understanding of this deregulation opens the way for testing and eventually transferring new possible therapies from bench to clinic.

REFERENCES

- I. Suhadolnik RJ, Reichenbach NL, Hitzges P, et al. Upregulation of the 2-5A synthetase/RNase L antiviral pathway associated with chronic fatigue syndrome. *Clin Infect Dis* 1994; 18 (Suppl. I):S96-S 104.
2. Sen GC. Viruses and interferons. *Annu Rev Microbiol* 2001; 55:255-281.
3. Der SD, Zhou A, Williams BRG, Silverman RH. Identification of genes differentially regulated by interferon α , β , or γ using oligonucleotide arrays. *Proc Natl Acad Sci USA* 1998; 95: 15623-15628.
4. Vojdani A, Choppa PC, Lapp CW. Down regulation of RNase L inhibitor correlates with upregulation of interferon-induced proteins (2-5A synthetase and RNase L) in patients with chronic fatigue immune dysfunction syndrome. *J Clin Lab Immunol* 1998; 50:1-16.
5. Grolleau A, Kaplan MJ, Hanash SM, Beretta L, Richardson B. Impaired translational response and increased protein kinase PKR expression in T cells from lupus patients. *J Clin Invest* 2000; 106: 1561-1568.
6. Schattner A, Cori Y, Hahn T, Sirota P. No evidence for autoimmunity in schizophrenia. *J Autoimmun* 1996; 9:661-666.
7. Bonnevie-Nielsen V, Martensen PM, Justesen J, et al. The antiviral 2',5'oligoadenylate synthetase is persistently activated in type I diabetes. *Clin Immunol* 2000; 96:11-18.
8. Flodstrom M, Maday A, Balakrishna D, Cleary MM, Yoshimura A, Sarvetnick N. Target cell defense prevents the development of diabetes after viral infection. *Nat Immunol* 2002; 3:373-382.
9. Player MR, Torrence PF. The 2-5A system: modulation of viral and cellular processes through acceleration of RNA degradation. *Pharmacol Ther* 1998; 78:55-113.
10. Natelson BH. A status report on chronic fatigue syndrome. *Environm Health Perspect* 2002; 110:673-677.
- II. De Meirleir K, Bisbal C, Campine I, et al. A 37 kDa 2-5A binding

protein as a potential biochemical marker for chronic fatigue syndrome. *Am J Med* 2000; 108:99-105.

12. Bastide L, Demetree E, Martinand-Mari C, Lebleu B. Interferon and the 2-5A/pathway. In: P. Englebienne & K. De Meirleir (Eds.), *Chronic Fatigue Syndrome, A Biological Approach*. 2002; (pp. 1-15). Boca Raton: CRC Press.

13. Hartmann R, Norby PL, Martensen PM, et al. Activation of 2'-5' oligoadenylate synthetase by single-stranded and double-stranded RNA aptamers. *J Biol Chem* 1998; 273:3236-3246.

14. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF- κ B by Toll-like receptor 3. *Nature* 2001; 413:732-738.

15. Ahmad-Nejad P, Hacker H, Rutz M, Bauer S, Vabulas RM, Wagner H. Bacterial CpG DNA and lipopolysaccharides activate Toll-like receptors at distinct cellular compartments. *Eur J Immunol* 2002; 32: 1958-1968.

16. Krieg AM. CpG motifs in bacterial DNA and their immune effects. *Annu Rev Immunol* 2002; 20:709-760.

17. Magnusson, M, Magnusson S, Vallin H, Ronnblom L, Alm GV. Importance of CpG dinucleotides in activation of natural IFN- α -producing cells by a lupus-related oligodeoxynucleotide. *Scand J Immunol* 2001; 54:543-550.

18. Carpten J, Nupponen N, Isaacs S, et al. Germline mutations in the ribonuclease L gene in families showing linkage with HPC 1. *Nat Genet* 2002; 30: 181-184.

19. Rokman A, Ikonen T, Seppala EH, et al. Germline alterations of the RNASEL gene, a candidate HPC I gene at Iq25, in patients and families with prostate cancer. *Am J Genet* 2002; 70: 1299-1304.

20. Englebienne P, Verhas M, Herst CV, De Meirleir K. Type I interferons induce proteins susceptible to act as thyroid receptor (TR) corepressors and to signal the TR for destruction by the proteasome: possible etiology for unexplained chronic fatigue.

Med Hypotheses 2002; in press.

21. Dong B, Silverman RH. 2-5A-dependent RNase molecules dimerize during activation. *J Biol Chem* 1995; 270:4133-4137.

22. Dong B, Silverman RH. Alternative function of a protein kinase homology domain in 2',5'-oligoadenylate dependent RNase L. *Nucleic Acids Res* 1999; 27:439-445.

23. Bisbal C, Martinand C, Silhol M, Lebleu B, Salehzada T. Cloning and characterization of a RNase L inhibitor. *J Biol Chem* 1995; 270: 13308-13317.

24. Englebienne P, Herst CV, D'Haese A, et al. Ribonuclease L inhibitor: a member of the A TP-binding cassette super family. In: P. Englebienne & K. De Meirleir (Eds.), *Chronic Fatigue Syndrome, A Biological Approach* 2002 (pp. 73-97). Boca Raton: CRC Press.

25. Zimmerman C, Klein KC, Kiser PK, et al. Identification of a host protein essential for assembly of immature HIV -I capsids. *Nature* 2002; 415:88-92.

26. Castelli J, Wood KA, Youle RJ. The 2-5A system in viral infection and apoptosis. *Biomed Pharmacother* 1998; 52:386-390.
27. Bisbal C, Silhol M, Laubenthal H, et al. The 2'-5' oligoadenylate RNase L/RNase L inhibitor pathway regulates both MyoD mRNA stability and muscle cell differentiation. *Mol Cell Biol* 2000; 20:4959-4969.
28. Le Roy F, Bisbal C, Silhol M, Martinand C, Lebleu B, Salehzada T. The 2-5A/RNase LIRLI pathway regulates mitochondrial mRNAs stability in IFN α -treated H9 cells. *J Biol Chem* 2001; 276:48473-48482.
29. Rusch L, Zhou A, Silverman RH. Caspase-dependent apoptosis by 2',5' oligoadenylate activation of RNase L is enhanced by IFN-beta. *J Interferon Cytokine Res* 2000; 20: 1091-1100.
30. Li XL, Blackford JA, Judge CS, et al. RNase-L-dependent destabilization of interferon-induced mRNAs. *J Biol Chem* 2000; 275:8880-8888.
31. D'Cunha J, Ramanujam S, Wagner RJ, Witt PL, Knight E Jr, Borden EC. In vitro and in vivo secretion of human ISG 15, an IFN-induced immunomodulatory cytokine. *J Immunol* 1996; 157:4100-4108.
32. Loeb KR, Haas AL. The interferon-inducible 15-kDa ubiquitin homolog conjugates to intracellular proteins. *J Biol Chem* 1992; 267:7806-7813.
33. Hamerman JA, Hayashi F, Schroeder LA, et al. Serpin 2a is induced in activated macrophages and conjugates to a ubiquitin homolog. *J Immunol* 2002; 168:2415-2423.
34. Li XL, Hassel BA. Involvement of proteasomes in gene induction by interferon and double-stranded RNA. *Cytokine* 2001; 14:247-252.
35. D'Cunha J, Knight E Jr, Haas AL, Truitt RL, Borden EC. Immunoregulatory properties of ISG 15, an interferon-induced cytokine. *Proc Natl Acad Sci USA* 1996; 93:211-215.
36. Malakhov MP, Malakhova OA, Kim KI, Ritchie KJ, Zhang DE. UBP43 (USP18) specifically removes ISG15 from conjugated proteins. *J Biol Chem* 2002; 277:9976-9981.
37. Malakhova O, Malakhov M, Hetherington C, Zhang DE. Lipopolysaccharide activates the expression of ISG 15-specific protease UBP43 via interferon regulatory factor 3. *J Biol Chem* 2002; 277:14703-14711.
38. Suhadolnik RJ, Peterson DL, O'Brien K, et al. Biochemical evidence for a novel low molecular weight 2-5A-dependent RNase L in chronic fatigue syndrome. *J Interferon Cytokine Res* 1997; 17:377-385.
39. Shetzline SE, Suhadolnik RJ. Characterization of a 2',5' oligoadenylate (2-5A)-dependent 37-kDa RNase L: azido photoaffinity labeling and 2-5A-dependent activation. *J Biol Chem* 2001; 276:23707-23711.
40. Shetzline SE, Martinand-Mari C, Reichenbach NL, et al. Structural and functional features of the 37-kDa 2-5A-dependent RNase L in chronic fatigue syndrome. *J Interferon Cytokine Res* 2002; 22:443-456.
41. Demetree E, Bastide L, D'Haese A, et al. Ribonuclease L proteolysis in peripheral blood mononuclear cells of chronic fatigue syndrome patients. *J Biol Chem* 2002; 277:35746-35751.

42. Roelens S, Herst CV, D'Haese A, et al. G-actin cleavage parallels 2-5A-dependent RNase L cleavage in peripheral blood mononuclear cells. Relevance to a possible serum-based screening test for deregulations in the 2-5A pathway. *J Chronic Fatigue Syndrome* 2001; 8:63-82.
43. Marie I, Blanco J, Rebouillat D, Hovanessian AG. 69-kDa and 100-kDa isoforms of interferon-induced (2'-5') oligoadenylate synthetase exhibit differential catalytic parameters. *Eur J Biochem* 1997; 248:558-566.
44. De Becker P, Roeykens J, Reynders M, McGregor N, De Meirleir K. Exercise capacity in chronic fatigue syndrome. *Arch Intern Med* 2000; 160:3270-3277.
45. Englebienne P, Herst CV, De Smet K, D'Haese A, De Meirleir K. Interactions between RNase L ankyrin-like domain and ABC transporters as a possible origin for pain, ion transport, CNS and immune disorders of chronic fatigue immune dysfunction syndrome. *J Chronic Fatigue Syndrome* 2001; 8:83-102.
46. Chaudhuri A, Watson WS, Peam J, Behan PO. The symptoms of chronic fatigue syndrome are related to abnormal ion channel function. *Med Hypotheses* 2000; 54:59-63.
47. De Becker P, McGregor N, De Smet K, De Meirleir K. Current advances in CFS therapy. In: P. Englebienne & K. De Meirleir (Eds.), *Chronic Fatigue Syndrome, A Biological Approach*. 2002; (pp. 229-263). Boca Raton: CRC Press.
48. Ushijima H, Rytik PO, Schacke H, Scheffer U, Muller WE, Schroder HC. Mode of action of the anti-AIDS compound poly(I).poly(C 12U (Ampligen): activator of 2',5' -oligoadenylate synthetase and double-stranded RNA-dependent kinase. *J Interferon Res* 1993; 13:161-171.
49. Vojdani A, Ohoneum M, Choppa PC, Magtoto L, Lapp CWo Elevated apoptotic cell population in patients with chronic fatigue syndrome: the pivotal role of protein kinase RNA. *J Intern Med* 1997; 242:465-478.