Brain Science on Chronic Fatigue

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Abstract
The sense of fatigue is one of the important bio-alarm systems like pain or fever. However, the neural and molecular mechanisms of fatigue remain unclear. Chronic fatigue syndrome (CFS), involving a long-lasting sensation of fatigue, seems to be a good model for studying these mechanisms underlying chronic fatigue sensation. Recently, to explore the neural and molecular mechanisms of fatigue/chronic fatigue and to investigate the pathogenesis of CFS, we organized a study group of Japanese investigators from various fields, such as virology, immunology, endocrinology, physiology, biochemistry, psychiatry, and neuroscience. From our recent results, CFS can be understood as a special condition based on abnormality of the psycho-neuro-endocrino-immunological system, with the distinguishing feature of CFS seeming to be the secondary brain dysfunction caused by several cytokines and/or autoantibodies.

Key words Chronic fatigue, Social stress events, Genetic background, Cytokines, Neurotransmitters

Introduction
In 1999, a Japanese study group supported by the Ministry of Health and Welfare of Japan (Leader: Dr. Teruo Kitani) investigated the incidence of fatigue of 3,015 Japanese residents based on their response to a questionnaire. From this study, it became clear that around 60% of Japanese people have felt fatigue and that the 37% had chronic fatigue (lasting longer than 6 months). Surprisingly, 5.1% of the people felt a deterioration of their ability to perform personal daily tasks, and 1.8% of the people had a loss of daily work-related activity because of chronic fatigue with unknown reason(s). Only 8 of 3,015 (0.26%) fulfilled the CFS criteria proposed by the CDC.1 Therefore, chronic fatigue is becoming not only an important medical problem but also a serious social problem because of its big economical impact. Economical deficit by chronic fatigue and chronic fatigue syndrome was calculated and estimated to be over 10 billion US dollars.

In view of this situation, our proposal for studying the neural and molecular mechanisms of fatigue sensation was adopted in 1999 by the Japanese Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japanese Government, as one of the projects of the Special Coordination Funds for Promoting Science and Technology, and then more recently since 2004, it was also adopted as the 21st Century COE Program “Formation of Scientific and International Base to Overcome Fatigue” to our Osaka City University from MEXT. The pathogenesis of CFS was also investigated by the investigators from various fields, such as virology, immunology, endocrinology, physiology, biochemistry, psychiatry and neuroscience, and its mechanism is now becoming a little clearer. In this paper, we report our recent results and propose a hypothesis for neural and molecular mechanisms resulting in chronic fatigue, which account for the relationship among each of the abnormalities found in CFS.

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Pathogenesis of CFS

Social stress events

It is well known that social-stress events frequently become the trigger for acute mental fatigue, and occasionally cause chronic fatigue. There are some reports indicating that stressful events are associated with the onset of CFS, but there are others giving data showing the opposite. Thus, the roles of stress in CFS were unclear. Therefore, we studied the social-stress events of 71 Japanese patients with CFS and 223 age-matched healthy controls by using a questionnaire. Social stress was investigated by counting the events described in the Social Readjustment Rating Scale reported by Holmes and Rahe. In the CFS group, the social-stress events were studied at the time of onset of CFS and during medical treatment. Most of the CFS patients in this study denied any association between social stress and their complaints. However, the average score in the CFS group was 8.3 at the time of onset and 6.0 at the time of medical treatment, which values were significantly higher than that for the healthy control group (4.4, \( P < 0.01 \), Mann-Whitney U test). Therefore, it became clear that most of the Japanese CFS patients were under much social stress with or without being aware of it. However, we should emphasize that this result does not mean CFS to be a psychological illness. As described later, stressful social events are related to abnormality of the psycho-neuro-endocrino-immunological system; and the secondary brain dysfunction caused by the abnormal production of several cytokines and/or autoantibodies might be a key feature of CFS.

Genetic background

When evaluating a stress-related disease, we should pay attention not only to the absolute magnitude or frequency of stressful events but also to the susceptibility to and resistance against stress, which factors are thought to be related to personal character or disposition. Indeed, when we studied the personality in the patients with CFS, most of them had a predisposition toward perfectionism and/or over-adaptation, which tendencies were not related to the existence or not of mental illness. We suspect that such predisposition might be related to genetic polymorphism of transporters and/or receptors of various neurotransmitters.

Recently we examined the polymorphism of the promoter region of the serotonin transporter (5-HTT) gene, which region affects the transcriptional efficiency of 5-HTT, in 78 CFS patients by performing PCR amplification of their blood genomic DNA. A significant increase in the frequency of longer (L and XL) allelic variants was found in the CFS patients compared to the controls both by genotype-wise and the allele-wise analyses (\( P < 0.05 \), Table 1). Efficiency in the transportation of 5-HTT is known to be higher with the L allele than with the S allele. There was no significant difference in 2 other 5-HT-related polymorphisms, i.e., the 5-HTT 

Table 1 Comparison of genotype distribution and allele frequencies between the CFS patients and the control subjects (Ref. 3)

<table>
<thead>
<tr>
<th></th>
<th>CFS</th>
<th>Controls</th>
<th>( \chi^2 ) test</th>
<th>Fisher’s exact test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>S/S</td>
<td>42 (54%)</td>
<td>39 (78%)</td>
<td>( \chi^2 = 7.887 )</td>
<td>( P = 0.031 )</td>
</tr>
<tr>
<td>L/S</td>
<td>32 (41%)</td>
<td>10 (20%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L/L</td>
<td>3 (4%)</td>
<td>1 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XL/L</td>
<td>1 (1%)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td>( \chi^2 = 7.233 )</td>
<td>( P = 0.012 )</td>
</tr>
<tr>
<td>S</td>
<td>116 (74%)</td>
<td>88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>39 (25%)</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XL/L</td>
<td>1 (1%)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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patient group and control group. Therefore, we speculate that the polymorphism within the 5-htr
5' upstream region is closely linked to CFS and may be a risk factor for this disorder.

When we used fluvoxamine maleate, one of the selective serotonin reuptake inhibitors, i.e., 5-HTT inhibitors, for the treatment of 39 Japanese patients with CFS, 11 patients withdrew from the treatment within 2 weeks because of the side effects such as nausea, increased fatigue, and a loss of thinking ability. However, the remaining 28 patients were given the inhibitor for more than 2 months. As a result, 2 of them were cured from CFS after the treatment, and 8 of them recovered enough to return to work. Therefore, serotonergic hypofunction was considered as one of the aspects concerning the pathogenesis of CFS. However, there is another possibility of the existence of polymorphisms of genes for transporters and/or receptors of other neurotransmitters, and such studies are currently on-going in our laboratory.

**Immunological abnormalities**

It is well known that the prevalence of past history of allergy is high in patients with CFS. Furthermore, CFS patients were reported to have many immunological abnormalities of various types, such as low natural killer cell function, abnormality of T cell population, elevated levels of several kinds of cytokines, the presence of antinuclear antibody, an increased level of immune complexes, and abnormality of the RNase-L pathway. Among these abnormalities, we are now focusing on the elevation of several kinds of cytokines, the presence of autoantibodies, and the abnormality of the RNase-L pathway.

It is also well known that flu-like symptoms are a common side effect of interferon (IFN) therapy; and elevated activity of 2',5'-oligoadenylate synthetase, an enzyme involved in, is frequently found in peripheral blood mononuclear cells from CFS patients. Therefore, much attention has been paid to the relationship between IFN and the pathogenesis of CFS. The abnormality of the RNase-L pathway is located in the downstream of the IFN pathway.

Recently Katafuchi et al., who was one of the members in our fatigue project, found a close association between the changes in the IFN-alpha mRNA content in the brain and immunologically induced fatigue. An intraperitoneal injection of a synthetic double-stranded RNA, poly I:C 3mg/kg, was given to rats to produce immunologically induced fatigue. The daily amounts of spontaneous running wheel activity decreased to ca. 40–60% of the pre-injection level until day 9, with normal circadian rhythm. Quantitative analysis of mRNA levels conducted by using the real-time capillary reverse transcriptase-polymerase chain reaction (RT-PCR) method revealed that IFN-alpha mRNA contents in the cortex, hippocampus, hypothalamic medial preoptic, paraventricular, and ventromedial nuclei were higher in the poly I:C group than in the saline and heat-exposed groups on day 7. These results suggest that brain IFN-alpha may play a role in the animal model for the immunologically induced fatigue mimicked with viral infection. They also found that the expression of 5-HTT mRNA in the brain was increased in this model and that treatment with a selective serotonin reuptake inhibitor (SSRI) was effective for blocking the decrement of the daily amount of spontaneous running wheel activity. Therefore, the relationship among viral infection, changes in cytokine production, and brain dysfunction is gradually becoming clearer.

Moreover, there is a possibility that the abnormalities of transforming growth factor beta (TGF-beta) are also deeply concerned with fatigue sensation. Inoue et al. found that intracranial administration of cerebrospinal fluid (CSF) from exercise-exhausted rats to naive mice produced a decrease in spontaneous motor activity, whereas CSF from sedentary rats had no such effect. This finding suggests the presence of a substance suppressing the urge for motion as a response to fatigue. Using a bioassay system, they found the level of TGF-beta in the CSF from exercise-fatigued rats to be increased; but there was no increase in the CSF from the sedentary rats. Furthermore, the injection of recombinant TGF-beta into the brains of sedentary mice elicited a similar decrease in spontaneous motor activity in a dose-dependent manner. These results suggest that TGF-beta might be involved in the fatigue upon exercise and thus suppresses spontaneous motor activity.

An elevated serum level of bioactive TGF-beta was also frequently found in patients with CFS and we also confirmed such an increase.
in the majority of Japanese CFS patients. TGF-beta was reported to inhibit the production of dehydroepiandrosterone sulfate (DHEA-S), which is known to regulate positively the activity of carnitine acetyltransferase, which catalyzes the transfer of free carnitine to acylcarnitine, especially the acetylcarnitine. We found that most Japanese CFS patients had a deficiency in DHEA-S and that in acetylcarnitine, and so the increase in TGF-beta would appear to be related to these abnormalities.

Additionally, the presence of auto-antibodies including antinuclear antibody is also thought to be an important key immunological abnormality involved in the pathogenesis of CFS. It is known that antinuclear antibody is frequently found in fatigued patients throughout the world who have various indefinite complaints. However, the role of these auto-antibodies in these patients is unclear.

Recently, using a sensitive radioligand assay Tanaka et al. examined the sera of CFS patients (n = 60), patients with autoimmune disease (n = 33), and healthy controls (n = 30) for auto-antibodies against various neurotransmitter receptors, i.e., recombinant human muscarinic cholinergic receptor 1 (CHRM1), mu-opioid receptor (OPRM1), 5-hydroxytryptamine receptor 1A (HTR1A), and dopamine receptor D2 (DRD2). The mean anti-CHRM1 antibody index was significantly higher in patients with CFS (P < 0.0001) and autoimmune disease (P < 0.05) than in healthy controls, and over a half of the patients with CFS (53.3%, 32/60) had anti-CHRM1 antibody. Antinuclear antibodies were also found in 56.7% (34/60) of the CFS patients, but their titers did not correlate with the activities of the above 4 auto-antibodies. The CFS patients with positive auto-antibodies against CHRM1 had a significantly higher mean score (1.81) of ‘feeling of muscle weakness’ than those negative for them (1.18) (P < 0.01). Higher scores on ‘painful lymph node,’ ‘forgetfulness,’ and ‘difficulty in thinking’ were also found in CFS patients with anti-CHRM1 antibodies than in those without them, but statistical significance was not reached. Anti-OPRM1 antibodies, anti-HTR1A antibodies, and anti-DRD2 antibodies were also found in 15.2, 1.7, and 5.0% of patients with CFS, respectively; but no significant relationship was found between the symptoms and existence of these antibodies. Since anti-CHRM1 antibody is also frequently found in patients with schizophrenic disorders, mood disorders, and other psychiatric disorders, it is not specific for CFS; but the autoimmune abnormalities in neurotransmitter receptors might cause the secondary brain dysfunction including CFS.

**Infection**

At the onset of CFS, patients frequently complained the flu-like symptoms such as headache, sore throat, fever, painful lymph node, myalgia, and althralgia. Mass outbreaks of CFS have also sometimes been reported throughout the world. Therefore, many investigators have tried to find pathogens or pathogenic organisms as candidates for causing CFS; and many viruses and microorganisms have been reported to be involved in the pathogenesis of CFS. Examples include various herpes viruses (Epstein-Barr (EB) virus, human herpes virus-6, herpes simplex virus, varicella zoster virus, and cytomegalovirus), influenza virus, retroviruses, coxackie B virus, Borna disease virus, hepatitis C virus, parvovirus, mycoplasma infection, and chronic rickettsial infections. Indeed, we have found some patients to acquire CFS after developing an acute infection such as mononucleosis caused by EB virus infection. However, the vast majority of pathogens or pathogenic organisms found in patients with CFS did not represent an initial acute infection, but rather a reactivation of various kinds of herpes viruses and/or chronic mycoplasma infections. These infections might be related to deterioration of immune function, but the infections themselves seem not to be so serious for health. The important point is that most complaints given by these patients stem from cytokines produced by the immune response to these pathogens or pathogenic organisms, which cytokines cause secondary brain dysfunction.

**Hypothalamo-pituitary-adrenal (HPA) dysfunction and metabolic abnormalities**

In 1991, Demitrack et al. reported the impaired activation of the HPA axis in patients with CFS; and thereafter several investigators addressed HPA dysfunction in patients with CFS, including lower basal plasma cortisol levels, reduced salivary cortisol levels, lower ACTH response in insulin tolerance test and psychosocial stress test, reduced ACTH responses to CRH, and prolonged...
suppression of salivary free cortisol in the low-dose dexamethasone suppression test.\textsuperscript{21–23} We also found that the majority of Japanese patients with CFS had a deficiency in serum DHEA-S.\textsuperscript{14} Serum DHEA-S is one of the most abundantly produced hormones secreted from the adrenal glands, and its physiological role is thought to be a precursor of sex steroids. However, DHEA-S itself was recently shown to have physiological properties, acting as a neurosteroid associated with such psychophysiological phenomena as memory, stress, anxiety, sleep, and depression. Therefore, the deficiency in DHEA-S might be related to the neuropsychiatric symptoms in patients with CFS. As described above, there is also a possibility that the DHEA-S deficiency is associated with the increased serum level of TGF-beta.

Recently, we also found that most Japanese patients with CFS showed a low level of serum acetylcarnitine, which well correlated with the rating score of fatigue,\textsuperscript{15} and that a considerable amount of the acetyl moiety of serum acetylcarnitine is taken up into the brain.\textsuperscript{24} As mentioned earlier DHEA-S is known to regulate the activity of carnitine acetyltransferase.\textsuperscript{13} Therefore, the decrease in DHEA-S might play an important role in endogenous acetylcarnitine deficiency in serum. Indeed, when we administered DHEA-S to patients with CFS, an apparent increase in serum acetylcarnitine was found. It was also found that the acetyl moiety taken up into the brain through acetylcarnitine is mainly utilized for the biosynthesis of glutamate.\textsuperscript{25} Thus, this metabolic abnormality (i.e., low uptake of acetylcarnitine into the brain) might cause some of the secondary brain dysfunction in CFS.

**Brain dysfunction**

Recent single-photon emission computed tomography (SPECT) studies\textsuperscript{26–28} using 99mTc-hexamethyl-propylene-amine oxime revealed that most CFS patients showed cerebral hypoperfusion in a variety of brain regions such as the frontal, temporal, parietal, and occipital cortices; anterior cingulate; basal ganglia; and brain stem, and suggested that the central nerve system (CNS) dysfunction might be related to the neuropsychiatric symptoms of CFS patients. To confirm these findings, we studied the regional cerebral blood flow (rCBF) in 8 CFS patients and 8 age- and sex-matched controls by use of \textsuperscript{15}O-labeled water (H\textsubscript{2}\textsuperscript{15}O) and positron emission tomography (PET), and found that the rCBF was lower in the CFS patient group than in the control group in the brain regions including the frontal, temporal, and occipital cortices, anterior cingulate; basal ganglia; and brain stem.\textsuperscript{25} These brain regions correspond to various neuropsychiatric complaints: autonomic imbalance, sleep disturbance, many kinds of pain, and the loss of concentration, thinking, motivation, and short-term memory. Therefore, our results from the first quantitative rCBF study done on CFS patients with PET are in good agreement with the data from the previous SPECT studies, and indicate that various neuropsychiatric complaints found in CFS patients might be related to dysfunction in these regions of the CNS.

Furthermore, when we studied the cerebral uptake of [2-\textsuperscript{11}C]acetyl-L-carnitine in the same 8 CFS patients and 8 age- and sex-matched normal controls by using PET, a significant decrease was found in several brain regions of the patients’ group, namely, in the prefrontal (Brodman’s area 9/46d) and temporal (BA21 and 41) cortices, anterior cingulate (BA24 and 33), and cerebellum.\textsuperscript{25} These findings suggest that the levels of neurotransmitters biosynthesized through acetylcarnitine might be reduced in some brain regions of chronic-fatigue patients and that this abnormality might be one of the keys to unveil the mechanisms of chronic-fatigue sensation.

More recently, using MRI, we found that patients with CFS have reduced gray matter (GM) volume in the bilateral prefrontal cortices.\textsuperscript{29} Furthermore, right-hemisphere GM volume correlated with subjects’ fatigue ratings.\textsuperscript{29} This is consistent with above-mentioned result that described a decrease of uptake of acetylcarnitine, maybe indicating a decrease in the biosynthesis of glutamate, in the prefrontal cortex. The prefrontal cortex might therefore be part of the neural underpinnings of fatigue.

We also studied 5-HT transporter (5-HTT) density in 10 patients with CFS and 10 age-matched normal controls by using PET with the radiotracer \textsuperscript{[11]}C]{(+)McN5652. Analysis using a statistical parametric mapping software (SPM99) revealed that the density of 5-HTT in the rostral subdivision of the anterior cingulate was significantly reduced in CFS patients.\textsuperscript{30} In addition, the density of 5-HTT of dorsal anterior cingulate...
was negatively correlated with the pain score. Therefore, an alteration in the serotonergic neurons in the anterior cingulate plays a key role in the pathophysiology of CFS.

These PET results on 5-HTT density seem to be apparently inconsistent with our results regarding the 5-HTT gene promoter polymorphism, where CFS patients could have a greater frequency of the L allele, which affords greater transporter efficiency. However, it might be the case that the reduction in 5-HTT density in CFS patients with L and XL allelic variants is less than that in CFS patients with S allelic variants. Since 5-HT synthesis in the brain is thought to deteriorate in patients with CFS, 5-HT deficiency in the synapses might be more serious in patients with L and XL allelic variants. If so, it is consistent with the finding that SSRI treatment is effective for some patients with CFS. To clarify the full particulars of brain dysfunction in patients with CFS, we are now studying the 5-hydroxy-L-tryptophan (5-HTP) uptake, L-DOPA uptake, and muscarinic acetylcholine receptor density by using PET. We could further report the results from these studies concerning brain dysfunction found in patients with CFS in the near future. But so far we propose the working hypothesis on the dysfunction in chronic fatigue, as shown in Fig. 1.

**Hypothesis: neuronal and molecular mechanisms leading to chronic fatigue**

It is becoming clear that various abnormalities found in CFS patients might not exist independently, but might be related to each other. That is, CFS can be understood to be a special condition based on the abnormality of the psycho-neuro-endocrino-immunological system caused by the psycho-social stress and some genetic components (Fig. 2). Under these conditions, a reactivation of various kinds of herpes virus infections and/or chronic mycoplasma infection might occur as a result of immune dysfunction, causing the abnormal production of several cytokines. A distinctive feature of CFS is thought to be the secondary brain dysfunction caused by the abnormal production of such cytokines.

As described above, the increase in TGF-beta might inhibit the production of DHEA-S, which inhibition might be related to the malmetabolism of acetyl-L-carnitine through the modulation of carnitine acetyltransferase activity. Indeed, when we administered DHEA-S to the patients...
with CFS in a double-blind study, an apparent increase in serum acetylcarnitine was found in the patients treated with DHEA-S. Therefore, one of the pathways leading to CFS may be described as follows: “increase of TGF-beta” → “decrease of DHEA-S” → “acetylcarnitine malmetabolism” → “deterioration of biosynthesis of glutamate in anterior cingulate” → “autonomic imbalance and prolonged fatigue.”

The abnormal production of IFN is another important pathway whose activation results in fatigue sensation in CFS. That is, “reactivation of various kinds of herpes virus infections or chronic mycoplasma infection” → “abnormal production of IFN in the brain” → “elevation of 5-HTT mRNA contents in the brain” → “5-HT deficiency in synapse” → “depression, chronic pain disorder, and prolonged fatigue.” Abnormal production of IFN is thought to trigger yet another pathway leading to CFS, that is, “abnormal production of IFN” → “elevation of 2’,5’-oligoadenylate synthetase activity” → “abnormality of RNase-L pathway” → “CNS dysfunction” → “various neuropsychiatric complaints.”

In this paper, we focused on TGF-beta and IFN, but tumor necrosis factor (TNF) might also be involved in fatigue sensation, since it is known that TNF is associated with the symptoms found in cachexia of patients with advanced cancer.6 There is a possibility that other kinds of cytokines such as IL-2, IL-4, IL-6, and IL-10 might also be involved in the secondary brain dysfunction in CFS. Furthermore, recent PET studies of ours have revealed that the brain dysfunction found in CFS involves not only abnormal serotonergic and glutamatergic system, but also abnormal dopaminergic system. There is a possibility that muscarinic cholinergic system might also be defective in patients with CFS.

Fig. 2 Hypothesis: neuro-molecular mechanism leading to chronic fatigue

Most complaints given by CFS patients might stem from cytokines produced by the immune response to pathogens or pathogenic organisms, which cytokines cause secondary brain dysfunction.
Therefore, even if a distinctive feature of CFS is summarized as the secondary brain dysfunction caused by abnormal production of cytokines, its pathogenesis is obviously heterogeneous. In addition, the auto-antibodies against neurotransmitter receptors might also be involved in the pathogenesis of CFS in some cases.

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References