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Increased cerebrospinal fluid complement C5 levels in major depressive disorder and schizophrenia

Takashi Ishii [a, c], Kotaro Hattori [a, b], Tomoko Miyakawa [a, b], Kentaro Watanabe [a], Shinsuke Hidese [a], Daimei Sasayama [a, g], Miho Ota [a], Toshiya Teraishi [a], Hiroaki Hori [a, e], Sumiko Yoshida [b, f], Akihiko Nunomura [c], Kazuyuki Nakagome [d], Hiroshi Kunugi [a, *]

[a] Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan
[b] Medical Genome Center, National Center of Neurology and Psychiatry, Tokyo, Japan
[c] Department of Neuropsychiatry, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi, Japan
[d] National Institute of Mental Health, National Center of Neurology and Psychiatry, Tokyo, Japan
[e] Department of Adult Mental Health, National Institute of Mental Health, National Center of Neurology and Psychiatry, Tokyo, Japan
[f] National Center of Neurology and Psychiatry Hospital, Tokyo, Japan
[g] Department of Psychiatry, Shinshu University School of Medicine, Matsumoto, Japan

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ABSTRACT

Inflammation has been implicated in a variety of psychiatric disorders. We aimed to determine whether levels of complement C5 protein in the cerebrospinal fluid (CSF), which may reflect activation of the complement system in the brain, are altered in patients with major psychiatric disorders. Additionally, we examined possible associations of CSF C5 levels with clinical variables. Subjects comprised 89 patients with major depressive disorder (MDD), 66 patients with bipolar disorder (BPD), 96 patients with schizophrenia, and 117 healthy controls, matched for age, sex, and ethnicity (Japanese). Diagnosis was made according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, criteria. CSF C5 levels were measured by enzyme-linked immunosorbent assay. CSF C5 levels were significantly increased in the patients with MDD ($p < 0.001$) and in the patients with schizophrenia ($p = 0.001$), compared with the healthy controls. The rate of individuals with an "abnormally high C5 level" (i.e., above the 95th percentile value of the control subjects) was significantly increased in all psychiatric groups, relative to the control group (all $p < 0.01$). Older age, male sex, and greater body mass index tended to associate with higher C5 levels. There was a significantly positive correlation between C5 levels and chlorpromazine-equivalent dose in the patients with schizophrenia. Thus, we found, for the first time, elevated C5 levels in the CSF of patients with major psychiatric disorders. Our results suggest that the activated complement system may contribute to neurological pathogenesis in a portion of patients with major psychiatric disorders.

1. Introduction

Current diagnostic systems for mental disorders rely upon the presentation of signs and symptoms, and thus do not adequately reflect the relevant neurobiological and behavioral systems [1]. There is an urgent need to elucidate molecular basis of mental disorders and to develop biomarkers that allow classification of psychiatric disorders based on the pathophysiology. In this context, investigation of the cerebrospinal fluid (CSF) might be one of the most promising approaches to detect molecules that contribute to the pathophysiology of psychiatric diseases within the brain, as CSF is in contact with the brain interstitial fluid over the surfaces of ventricles, brain, and spinal cord; therefore, molecules released from brain cells can directly diffuse into the CSF. Indeed, CSF biomarkers have already been established for some neurological diseases, such as tau, phosphorylated tau, and β-amyloid proteins, which diagnose Alzheimer's disease with a high (~90%) sensitivity and specificity [2].

There is a growing amount of evidence that supports increased levels of pro-inflammatory cytokines in the blood of patients with

* Corresponding author. 4-1-1, Ogawahigashi, Kodaira, Tokyo, 187-8502, Japan.
E-mail address: hkunugi@ncnp.go.jp (H. Kunugi).

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Abbreviations

BBB	blood-brain barrier
BMI	body mass index
BPD	bipolar disorder
CNS	central nervous system
CPeq	chlorpromazine-equivalent dose
CSF	cerebrospinal fluid
ELISA	enzyme-linked immunosorbent assay
GRID-HAMD-17	GRID-Hamilton Depression Rating Scale, 17-item version
IL-6	interleukin-6
IMIeq	imipramine-equivalent dose
MDD	major depressive disorder
MINI	Mini International Neuropsychiatric Interview
PANSS	Positive and Negative Syndrome Scale
YMRS	Young Mania Rating Scale

schizophrenia and major depressive disorder (MDD) [3,4]. We previously reported increased interleukin-6 (IL-6) levels in the blood of patients with schizophrenia [4]. Contrary to the traditional view that the brain is an immunologically privileged site that is shielded behind the blood-brain barrier (BBB), studies in the past 20 years have uncovered complex interactions among the immune system, systemic inflammation, and brain functions, which can lead to changes in mood, cognition, and behavior [5]. As stated above, we have reported increased IL-6 in the CSF of patients with schizophrenia, as well as in patients with MDD, relative to healthy controls [6]; these findings were further supported by a recent meta-analysis [7]. Additionally, we have reported increased CSF fibrinogen levels in patients with MDD [2]. Fibrinogen is a coagulation factor which activates microglia, thereby inducing neuroinflammation and negatively affecting the brain [8].

A major aspect of the innate immune system is the complement system. In general, complement activation occurs via four pathways: the classical, alternative, mannan-binding lectin, and extrinsic protease pathways [9,10]. These pathways converge upon the cleavage of complement C5 into complement fragments C5a and C5b by C5 convertase, which is the final step of the complement activation. C5a is the most potent and stable of the anaphylatoxins, while C5b is an initial element in the formation of the membrane attack complex. Notably, the complement system also plays a role in synapse elimination/pruning, which is essential to the development of a precise neuronal network [11,12]. A recent study on adult Swedish twins enriched for schizophrenia reported that peripheral messenger RNA (mRNA) expression levels of two complement genes (C5, SERPING1) in peripheral blood mononuclear cells were associated with decreased superior frontal cortical thickness, which may be involved in the pathogenesis of schizophrenia [13]. A number of studies, including ours, have reported structural and functional changes in volume, gray matter, white matter, and functional activity, within the frontal lobe of schizophrenia patients [14,15]. Since C5 is involved in the final step of the activation of the complement system, we hypothesized that C5 levels would be altered in the brain of patients with schizophrenia.

The aim of the present study was to determine whether CSF complement C5 levels were altered in patients with major psychiatric disorders, i.e., MDD, bipolar disorder (BPD), and schizophrenia, compared with healthy controls. We further examined the possible association of CSF C5 levels with clinical variables.

2. Material and methods

2.1. Participants

Subjects comprised 89 patients with MDD (age: 43.8 ± 10.4 years; 43 males), 66 patients with BPD (43.7 ± 12.3; 32 males), 96 patients with schizophrenia (40.1 ± 10.3; 58 males), and 117 healthy controls (42.5 ± 15.3; 66 males), matched for age, sex, and ethnicity (Japanese). Patients with BPD included 23 patients with bipolar I and 43 with bipolar II disorder. All participants were recruited at the National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan, via advertising at the NCNP hospital, on our website, and in local free magazines. All participants underwent a structured interview using the Japanese version of the Mini International Neuropsychiatric Interview (MINI) [16], administered by a trained psychologist or board-certificated psychiatrist. Diagnosis was made according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition Criteria (American Psychiatric Association, 1994), on the basis of the MINI, additional unstructured interviews, and medical record (if available). Healthy controls were volunteers without a current or past history of contact with psychiatric services. Participants were excluded if they had a medical history of central nervous system (CNS) diseases, severe head injury, substance abuse, or mental retardation. The study protocol was approved by the ethics committee at the NCNP; the study was conducted according to the Declaration of Helsinki (World Medical Association, 2000). Written informed consent was obtained from every participant.

2.2. Clinical assessments

The Positive and Negative Syndrome Scale (PANSS) was used to assess symptoms in patients with schizophrenia [17]. The GRID-Hamilton Depression Rating Scale, 17-item version (GRID-HAMD-17), was used to evaluate depressive symptoms in patients with MDD or BPD [18]. The Young Mania Rating Scale (YMRS) was used to evaluate manic symptoms in patients with BPD [19]. Daily doses of antipsychotics were converted into chlorpromazine-equivalent doses (CPeqs) and doses of antidepressants were converted into imipramine-equivalent doses (IMIeqs), using the published guidelines [20]. The status of medication use was recorded at the time of lumbar puncture.

2.3. Lumbar puncture

After neurologic examinations, each participant underwent local anesthesia, followed by a lumbar puncture at the L3-4 or L4-5 interspace using an atraumatic pencil point needle (Universe 22G, 75 mm, Unisis Corp., Tokyo, Japan). CSF was collected using a low protein absorption tube (PROTEOSAVE SS 15 mL Conical tube, Sumitomo Bakelite Co., Tokyo, Japan) and immediately placed on ice. The CSF was centrifuged ($4000 \times g$ for 10 min) at 4 °C, then the supernatant was dispensed into 0.5 mL aliquots in low protein absorption tubes (PROTEOSAVE SS 1.5 mL Slim tube, Sumitomo Bakelite Co.) and stored in a deep freezer (−80 °C) until use.

2.4. Enzyme-linked immunosorbent assay (ELISA)

We used a Human Complement C5 ELISA Kit (ab125963, Abcam Japan, Tokyo, Japan). The CSF samples were diluted 1:100, using the diluent attached to the kit. Prior to the experiment, the experimenter (T.I) validated the reproducibility of the ELISA at a level of 4% coefficient of variation, using the kit.

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