Clinical Immunology (2015) 157, 114–120

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

BRIEF COMMUNICATION

Distinguishing the cerebrospinal fluid cytokine profile in neuropsychiatric systemic lupus erythematosus from other autoimmune neurological diseases

Kunihiro Ichinose[a,*], Kazuhiko Arima[b], Takeshi Ushigusa[a], Ayako Nishino[a], Yoshikazu Nakashima[a], Takahisa Suzuki[a], Yoshiro Horai[a], Hideki Nakajima[d], Shin-ya Kawashiri[b], Naoki Iwamoto[a], Mami Tamai[a], Hideki Nakamura[a], Tomoki Origuchi[c], Masakatsu Motomura[d], Atsushi Kawakami[a]

[a] Department of Immunology and Rheumatology, Nagasaki University Graduate School of Biomedical Sciences, Japan
[b] Department of Public Health, Nagasaki University Graduate School of Biomedical Sciences, Japan
[c] Department of Rehabilitation Sciences, Nagasaki University Graduate School of Biomedical Sciences, Japan
[d] Department of Clinical Neuroscience and Neurology, Nagasaki University Graduate School of Biomedical Sciences, Japan

Received 3 September 2014; accepted with revision 24 January 2015
Available online 3 February 2015

KEYWORDS
Neuropsychiatric systemic lupus erythematosus;
Cytokine profiles;
Cerebrospinal fluid;
Weighted-voting algorithm

Abstract
Neuropsychiatric systemic lupus erythematosus (NPSLE) is a serious complication in SLE. Although the mechanism of NPSLE remains unclear, cytokines and chemokines are considered to be involved in their pathogenesis. Here we used Bio-Plex Pro assays to examine 27 types of cytokines and chemokines in the cerebrospinal fluid (CSF) of 32 NPSLE patients. We used the CSF of 20 patients with multiple sclerosis (MS) and 22 patients with neuromyelitis optica (NMO) as a disease control group. Fourteen of 27 cytokines/chemokines were significantly higher in the NPSLE patients compared to the MS/NMO patients. We could identify six "minimum predictive markers" by using a weighted-voting algorithm that could distinguish NPSLE from MS and NMO: interleukin (IL)-17, IL-2, interferon (IFN)-γ, IL-5, basic fibroblast growth factor (FGF)-basic and IL-15. The determination of various types of CSF cytokine profiles may contribute to the diagnosis of NPSLE and may help elucidate the mechanisms underlying this disease.

* Corresponding author at: Department of Immunology and Rheumatology, Nagasaki University Graduate School of Biomedical Sciences, Japan, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. Fax: +81 95 849 7270.
E-mail address: kichinos@nagasaki-u.ac.jp (K. Ichinose).

http://dx.doi.org/10.1016/j.clim.2015.01.010
1521-6616/

1. Introduction

Neuropsychiatric systemic lupus erythematosus (NPSLE) syndromes involve both the central and peripheral nervous systems. Despite advances in the understanding of the immunopathogenic and clinical aspects of SLE, NPSLE remains a diagnostic and therapeutic challenge [1]. Cytokines and chemokines are considered biomarkers and therapeutic targets in NPSLE. Of note, abnormalities in cerebrospinal fluid (CSF) have been reported in patients with NPSLE. Increased levels of proinflammatory cytokines and chemokines have been reported in the CSF of patients with NPSLE, including cytokines such as interleukin (IL)-6, IL-8, IL-10, tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), C-C motif ligand (CCL)2/monocyte chemoattractant protein-1 (MCP-1) and C-X-C motif ligand (CXCL) 10/inducible protein-10 (IP-10) [2,3]. Among these, CSF IL-6 is major cytokine for the diagnosis of NPSLE. IL-6 is a proinflammatory cytokine secreted by immune cells and activated astrocytes, with a wide variety of functions. The sensitivity and specificity of the diagnosis of lupus psychosis were 87.5% and 92.3%, respectively, at the cut-off value of 4.3 pg/mL [4].

Multiple sclerosis (MS) and neuromyelitis optica (NMO) are chronic autoimmune inflammatory diseases affecting the central nervous system (CNS). Disruption of the blood–brain barrier (BBB) is a known mechanism of the disease process in these two CNS diseases. MS and NMO are also considered T cell-mediated autoimmune diseases, and both the Th1/Th2 balance and Th17 cells play an important role in the pathogenesis [5]. Elevated CSF IL-6 and IL-8 levels in NMO patients have also been reported [6]. CSF IL-6 and IL-8 levels are significantly higher in patients with NMO than in patients with MS [6]. Similar cytokines/chemokines have been evaluated in NPSLE, MS and NMO but the therapeutic strategy and management are quite different among these three diseases.

In this study we evaluated multiple cytokines, chemokines and growth factors in NPSLE compared to MS and NMO as disease controls. We found a specific combination of cytokines, chemokines and growth factors in NPSLE that can be distinguished from the profiles of the other two diseases. This analysis might help clarify the mechanism of NPSLE caused by inflammation and may provide an important resource for pharmaceutical developments.

2. Methods

2.1. Study design and patients

We studied 32 patients who were admitted to Nagasaki University Hospital in a 7-year period from 2006 through 2013 and fulfilled at least four of the 11 revised criteria of the American College of Rheumatology (ACR) for the classification of SLE [7]; they were all diagnosed with NPSLE by rheumatologists and psychiatrists. Neuropsychiatric manifestations showing psychiatric symptoms such as mood disorder, anxiety disorder, psychosis, acute confusional state, or cognitive dysfunction were evaluated by a psychiatrist and classified according to the ACR nomenclature and case definitions for NPSLE [8].

All information about clinical symptoms and laboratory data were reviewed retrospectively using the patients' medical records. The patients' age, gender, clinical events, results of serum laboratory tests, the CSF analysis, brain magnetic resonance imaging (MRI), and single photon emission computed tomography (SPECT) and their diagnoses and treatment were all analyzed.

As disease controls, we used samples from 20 relapsing remitting MS (RRMS) patients, samples from 22 NMO patients, 11 normal pressure hydrocephalus (NPH) patients, and 16 viral meningitis (VM) patients from the Department of Clinical Neuroscience and Neurology, Nagasaki University Hospital. For the diagnosis of NMO, we defined NMO spectrum disorder (NMOSD) based on the revised NMO criteria [9]. All of the NMO patients were positive for anti-AQP4 antibodies in sera. CSF of NPH patients were used for non-autoimmune, non-inflammatory neurological controls and VM patients were used for positive controls. The protocol was approved by the Institutional Review Board of the Nagasaki University Hospital.

2.2. Multiplex cytokine bead assay

We performed a multiplex cytokine bead assay using undiluted CSF supernatants and the Bio-Plex Pro Human Cytokine Group I 27-Plex Panel analyzed with a Bio-Plex® MAGPIX™ Multiplex Reader (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. CSF samples were centrifuged within 30 min at 1500 rpm at 4 °C for 5 min, and the liquid phase of the CSF was stored at −80 °C until use. The levels of 27 cytokines/chemokines and growth factors in the liquid phase of the CSF, namely, IL-1β, IL-1 receptor antagonist (IL-1ra), IL-2, IL-4, IL-5, IL-6, IL-7, CXCL8/IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A, basic fibroblast growth factor (FGF)-basic, CCL11/eotaxin, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), IFN-γ, IP-10, CCL2/MCP-1, CCL3/macrophage inflammatory protein (MIP)-1α, CCL4/MIP-1β, platelet-derived growth factor (PDGF)-BB, CCL5/regulated on activation, normal T cell expressed and secreted (RANTES), TNF-α, and vascular endothelial growth factor (VEGF) were measured as described [10,11].

The cytokine/chemokine/growth factor concentrations were calculated based on the respective standard curve for each cytokine/chemokine/growth factor concentration of the standards assayed in the same manner as the CSF samples. The detection limit for each molecule was determined by the recovery of the corresponding standard, and the lowest values with more than 70% recovery were set as the lower detection limits. All samples were analyzed in duplicate.

2.3. Construction of diagnostic systems

Next, we selected which of the 27 cytokines/chemokines/growth factors were useful markers for distinguishing NPSLE from MS and NMO. The weighted-voting (WV) algorithm was used as described [12,13]. The ranking of the 27 cytokines/chemokines for this algorithm was based on the signal-to-noise ratio (SNR). For each marker set of 27