Clinica Chimica Acta 484 (2018) 26–31

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Proteomic approach to profiling immune complex antigens in cerebrospinal fluid samples from patients with central nervous system autoimmune diseases

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ARTICLE INFO

Keywords:
Immune complexome analysis
Immune complex
Tandem mass spectrometry
CNS autoimmune diseases

ABSTRACT

Background: Immune complexes (ICs) may clearly reflect immunological abnormalities caused by disease, especially for autoimmune diseases. Although ICs have been detected in cerebrospinal fluid (CSF) from patients with CNS autoimmune diseases, identities of antigens in such ICs have not been comprehensively determined.
Methods: We used immune complexome analysis, in which nano-liquid chromatography-tandem mass spectrometry is employed to comprehensively identify antigens incorporated into ICs in biological fluids, to characterize ICs in CSF samples from patients with CNS autoimmune diseases, and to find disease-specific IC antigen to a certain CNS autoimmune disease. Also, we compared the IC antigens we identified with the reported CSF proteome or with the published plasma proteome to examine if the method is distinguished from the conventional CSF proteome analysis.
Results: We identified 176 antigens in 78 CSF samples. We then assessed the overlaps among these antigens, the CSF proteome, and the plasma proteome; 140 of the 176 antigens were found to be exclusively detected by our method. Notably, IC-associated suprabasin in CSF was 100% specific to neuropsychiatric systemic lupus erythematosus (NPSLE).
Conclusions: This report is the first to comprehensively identify the antigens incorporated into ICs in CSF. There was limited overlap between the antigens we identified and the CSF proteome or the plasma proteome; therefore, our method can be distinguished from the conventional CSF proteome analysis. Although the sensitivity of disease-specific IC-antigens detected in immune complexome analysis screening, the sensitivity may be improved by developing an ELISA method specifically for detecting the ICs. Immune complexome analysis of CSF may be a new and promising path to biomarker discovery for diagnosis and study for CNS autoimmune diseases.

1. Introduction

The blood-brain barrier (BBB) is a multicellular vascular structure that separates the central nervous system (CNS) from peripheral blood circulation. The BBB comprises endothelial cells that have continuous intercellular tight junction, regulates influx and efflux transport, and protects the CNS from toxins and pathogens. This barrier also limits the cells and macromolecules that enter into cerebral circulation. However, multiple studies demonstrated that this strictly regulated barrier is compromised in several CNS diseases; extravasation of lymphocytes and serum proteins through the BBB can occur in patients who have inflammation, an infection, or both [1–3].

Abbreviations: AD, Alzheimer's disease; BBB, blood-brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; ICs, immune complexes; nano-LC-MS/MS, nano-liquid chromatography-tandem mass spectrometry; MS, multiple sclerosis; NMO, neuromyelitis optica; NPSLE, neuropsychiatric systemic lupus erythematosus; RA, rheumatoid arthritis; SBSN, suprabasin

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https://doi.org/10.1016/j.cca.2018.05.026
Received 16 March 2018; Received in revised form 23 April 2018; Accepted 11 May 2018

B cells contribute to the pathogenesis of CNS autoimmune diseases, which is indicated by local production of antibodies within the CNS [4,5], by damage of the CNS tissue by antibody and complement [6] and by the therapeutic effects of plasmapheresis or anti-CD20 monoclonal antibody [7,8]. Flach et al. reported that myelin-specific antibodies produced by autoreactive B cells after activation in the periphery diffused into the CNS together with the first invading pathogenic T cells [9]. On the other hand, BBB disruption, a critical step in pathogenesis of CNS autoimmunity, occurs antigen-specifically in brain endothelial cells [10–13]. Therefore, identifying specific CNS autoantigens is crucial for understanding the pathological processes of CNS autoimmune diseases.

Immune complexes (ICs) are produced when antigens bind with antibodies. Importantly, the identification of antigens in ICs might be different from identification of free antigens because ICs are the direct products of immune responses and clearly reflect immunological abnormalities caused by diseases, and IC deposits on a tissue can activate the complement pathway and consequently trigger inflammation. In fact, the presence of ICs was observed in cerebrospinal fluid (CSF) of patients with neuropsychiatric SLE (NPSLE) or multiple sclerosis (MS) which are one of CNS autoimmune diseases, or an infectious CNS disorder [5,14–20]. However, the identities of IC-associated antigens in CSF of patients with neurological diseases have never been comprehensively examined because tools for comprehensive identification of ICs-antigens are lacking [21], although only a few antigens from ICs have been identified by not comprehensive but selective detection methods for each antigen in CSF of patients with MS, *Borrelia bungdoriferi* or spinal cord schistosomiasis [5,19,20].

In order to comprehensively identify and profile constituent antigens in ICs, we developed a proteomic strategy, designated immune complexome analysis, in which ICs are separated from whole serum and then subjected to direct tryptic digestion and nano-liquid chromatography-tandem mass spectrometry [22]. We have successfully used this method to identify disease-specific IC antigens in the sera of patients with autoimmune diseases [22–25], infectious diseases [26,27] and cancer [28]. Therefore, profiling of IC-associated antigens in CSF by immune complexome analysis might provide insights into pathophysiology of CNS autoimmune diseases and other neurological diseases, and such analyses could form the basis for novel diagnostic and treatment strategies for these diseases.

Here, we first time performed immune complexome analysis of CSF samples from patients with a CNS autoimmune disease—NPSLE, MS or neuromyelitis optica (NMO)—or a non-autoimmune disease —Alzheimer's disease (AD) or Hashimoto's encephalopathy —and samples from healthy donors to comprehensively identify IC-associated antigens in CSF and to find disease-specific antigens among these IC-associated antigens. Additionally, we studied the overlap between the IC-associated antigens we detected in CSF and CSF proteome or plasma proteome to examine if immune complexome analysis of CSF explores a new path for discovery of disease-specific or pathogen-specific markers in CSF.

2. Materials and methods

2.1. Patients

CSF samples were collected from each patient; 74 patients with NPSLE (n = 26; 20–50 years; 26 female), MS (n = 15; 28–70 years; 10 female), NMO (n = 16; 29–80 years; 12 female), Hashimoto's encephalopathy (n = 7; 65–88 years; 5 female), or AD (n = 10; 53–80 years; 7 female) at Nagasaki University Hospital who fulfill the following criteria; American College of Rheumatology (ACR) nomenclature and case definitions for NPSLE [29], McDonald criteria for MS [30] and Wingerchuk criteria for NMO [31], or NINCDS-ADRDA Work Group (AD). Diagnostic guidelines for Hashimoto's encephalopathy have not been published. The clinical diagnostic consensus for Hashimoto's encephalopathy that is used in Japan was used for inclusion of each Hashimoto's encephalopathy case, and the clinical findings and clinical course of each case was typical. CSF from healthy donors (n = 4; 40–90 years; no female) were purchased from Analytical Biological Services (Wilmington, DE, USA). Each CSF sample was collected by performing a lumbar puncture and was stored at −80 °C before analysis. Each CSF sample was subjected to replicate analyses. All the experiments were performed in accordance with the Helsinki Declaration and with approval from the institutional ethics committees of the Graduate School of Biomedical Sciences, Nagasaki University. Written informed consent was obtained from each patient.

2.2. Immune complexome analysis

ICs in CSF were purified by magnetic beads with immobilized Protein G (PureProteome®, Millipore, Darmstadt, Germany). Beads (40 μl) were washed with 500 μl of phosphate-buffered saline (PBS, Wako Pure Chemicals, Osaka, Japan) and incubated with 10 μl of CSF diluted with PBS (1,9, v/v) for 30 min with gentle mixing. The beads with bound ICs were recovered with a magnet and washed three times with 500 μl of PBS. The beads were resuspended in 100 μl of 10 mM dithiothreitol (in 25 mM ammonium bicarbonate, Wako) and incubated at 56 °C for 45 min; then, 100 μl of 55 mM iodoacetamide (in 25 mM ammonium bicarbonate, Tokyo Chemical Industry, Tokyo, Japan) were added and the mixture was incubated at room temperature for 30 min in the dark. Subsequently, trypsin (in 0.05% acetic acid, Promega, Madison, WI, USA) was added, and the mixture was incubated overnight at around pH 8 and at 37 °C. Trifluoroacetic acid (10%, Nacalai Tesque, Kyoto, Japan) was added to stop the digestion, and the supernatant was recovered. Finally, the volume of this mixture was reduced to approximately 80 μl using reduced pressure. The peptide mixture (3 μl) was subjected to a nano-LC-electrospray ionization-tandem MS (LTQ-XL, Thermo Fisher Scientific, Waltham, MA, USA) equipped with the custom nanoLC system consisting of a LC-20AD LC pump (Shimadzu, Kyoto, Japan) with LC flow splitter (Accurate, Dionex, Sunnyvale, CA, USA) and an HCT PAL autosampler (CTC Analytics, Zwingen, Switzerland). The sample was loaded onto a nano-precolumn (300 μm i.d. × 5.0 mm, L-C-18, Chemicals and Evaluation and Research Institute, Tokyo, Japan) in the injection loop. Peptides were separated by a nano HPLC column (75 μm i.d. × 15 cm, Acclaim PepMap100C18, 3 μm, Dionex) with gradient elution and ion-sprayed into MS with a spray voltage from 1.2 to 2.0 kV. The mass spectrometer was configured to optimize the duty cycle length with the quality of data acquired by progressing from a full scan of the sample to three tandem MS scans of the three most intense precursor masses (as determined by Xcaliber® software [Thermo Fisher Scientific] in real time). MS/MS data were extracted using Proteome Discoverer v.3.3 (Thermo Fisher Scientific). Spectra were searched against a human subdatabase from the public non-redundant protein database of International Protein Index version 3.84 presented by The European Bioinformatics Institute using the following search parameters: mass type = monoisotopic precursor and fragments; enzyme = trypsin (KR); enzyme limits = full enzymatic cleavage allowing up to 2 missed cleavages; peptide tolerance = 2.0 amu; fragment ion tolerance = 1.0 amu; static modification = C (carbamidomethylation); differential modifications = M (oxidation), N, and Q (deamidation). The filter criteria (single, double, and triple charge peptides with a correlation factor [XCorr] and protein probability [P]) were adjusted maintaining the empirically determined protein false discovery rate at 5%. At the beginning of each day's measurement, the performance of nano-LC-MS/MS system was checked by confirming the sequence coverage of bovine serum albumin peptides (more than 70%).

3. Results

Using immune complexome analysis, we identified 176 IC-associated antigens in CSF samples from 1) patients with one of four CNS autoimmune diseases, 2) patients with AD or Hashimoto's