Full-length research article

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

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Nonstandard 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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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].

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 ºC 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 ºC. 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. x 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. x 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 atomic mass units; fragment ion tolerance = 1.0 atomic mass unit; 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 encephalopathy, or 3) healthy donors. In supplementary table 1, we express how often each antigen were detected in patients with a certain disease as a percentage of the total number of patients with the disease. Representative total ion chromatogram in immune complexome analysis of CSF sample is shown in Fig. 1 with that of serum sample.

To determine whether immune complexome analysis was distinct from conventional CSF proteome analysis, we assessed the overlaps among the 176 IC-associated antigens identified in CSF, the CSF proteome, and the plasma proteome (Fig. 2). Here, we used the CSF proteome characterized from the proteins comprehensively detected in healthy individuals who had no neurologic symptoms [32], while we used the plasma proteome published by Human Proteome Organization [33]. Initially, we compared our dataset (176 protein antigens) with the CSF proteome dataset (2628 proteins) and found that only 26 of the

185 protein antigens were also in the CSF proteome. Next, we compared the 176 IC-associated
186 CSF antigens with the 3020 proteins in the plasma proteome dataset and found that only 18
187 proteins were in both groups. Also, 8 antigens were found in all three groups; 140 of the 176
188 antigens were found to be exclusively detected by our method. Specifically, apolipoproteins,
189 complements, dermcidin, fibulin and desmplakin were detected in all three groups;
190 hemoglobins and uncharacterized proteins were detected in both the CSF immune
191 complexome and the CSF proteome; angiotensinogen, desmoglein, nuclear mitotic apparatus
192 protein, Rho GTPase-activating protein, A-kinase anchor protein and golgin were detected in
193 both the CSF immune complexome and the plasma proteome; all of these antigens were
194 randomly detected among the diseases and healthy donors

195 In addition to the above-used normal CSF proteome, we also compared previously
196 reported disease-associated CSF proteins— proteins elevated in cases of MS, clinically
197 isolated syndrome, encephalomyelitis, Perkinson's disease, or AD [34] —with the 140
198 antigens specific to the CSF immune complexome and with the 26 antigens that were found in
199 both the CSF proteome and the CSF immune complexome. Of the 140 CSF immune
200 complexome-specific antigens, only 6 (4.3%) were among the previously reported
201 disease-associated CSF proteins for MS, and they were 69 kDa protein, calmodulin,
202 fibrinogen, complement factor H, albumin and uncharacterized protein; of the 26 antigens that
203 were also included in the CSF proteome, 6 (23%) were among the previously reported
204 disease-associated CSF proteins.

205 Among the 140 immune complexome-specific antigens identified, two antigens

(suprabasin isoform 1 precursor and Isoform 7 of Nesprin-1) were 100% specific to NPSLE, and three (Isoform B of Fibulin-1, Isoform 10 of Fibronectin, and Isoform 12 of Fibronectin) were 100% specific to NMO. Of the disease-specific antigens, IC-associated suprabasin (SBSN) were found in 9 of 26 NPSLE patients (35% (9/26); 95% CI, 17%-57%) and appeared more sensitive than the others (Isoform 7 of Nesprin-1, 7.7% (2/26); Isoform B of Fibulin-1, Isoform 10 of Fibronectin and Isoform 12 of Fibronectin, 13% (2/16)). The antigens selectively detected in one or two disease groups are summarized with sensitivity and specificity in Table 1.

4. Discussion

Different proteomic approaches have been used to characterize the CSF proteome of healthy individuals or those of patients with AD, MS, or Parkinson's disease [34-41]. Zougman *et al.* published the first comprehensive study of the CSF proteome of healthy donors, and they identified 798 proteins [39]. Also, Schutzer *et al.* characterized the CSF proteome from healthy individuals who had no neurologic symptoms and compared this CSF proteome with the normal plasma proteome; they used high abundant protein (e.g. albumin and globulin) depletion and strong-cation exchange fractionation [32]. They identified 2630 proteins in the CSF proteome; 56% of these are CSF-specific and were not found in plasma proteome, which comprises 3654 proteins [32]. However, a comprehensive analysis of ICs in CSF from healthy individuals or from patients has never been performed. Several researchers have described partial analyses of ICs in CSF of patients with neurological diseases; these analyses involved

C1q binding assays, polyethylene glycol precipitation tests, ELISA or immunoblotting [5, 15-17, 19, 20]; however, these studies did not comprehensively identify the antigens that form ICs with the corresponding antibodies.

Here, we describe the first comprehensive identification of IC-associated antigens in CSF (Supplementary table 1). We used the CSF proteome comprising 2628 proteins described by Schutzer *et al.* [32] as one reference dataset; this dataset represents the most comprehensive single study of healthy donors without neurological symptoms, and we used the published Human Proteome Organization plasma proteome dataset of 3020 proteins [33] as another reference dataset. The amount of total protein in CSF is extremely low [42]; nevertheless, low-abundance proteins in CSF may still be masked by high-abundance proteins, and Schutzer *et al.* had used immunoaffinity depletion to remove highly abundant proteins from CSF; they then comprehensively identified the low-abundance CSF proteins to generate a CSF proteome dataset [32]. Notably, only 15% of the 176 protein antigens we identified were also in such CSF proteome dataset; therefore, immune complexome analysis selectively recovered ICs in CSF and identified the constituent antigens, and our method can be distinguished from the conventional CSF proteome analysis. On the other hand, some peripheral blood proteins can cross the BBB and enter cerebral circulation by a simple diffusion mechanism as a function of their molecular size. It had been generally believed that the majority of proteins in CSF originate from peripheral blood [43]. However, Zougman *et al.* reported that the CSF proteome has only partial overlap with the plasma proteome and that the CSF proteome derives from local protein sources not just from blood circulation [39]. In

our analysis, only 18 of 176 IC-associated antigens were also found in the plasma proteins. This indicates that the BBB limits peripheral ICs enter the brain, which was confirmed by our comparison of total ion chromatograms of CSF and serum samples that had been subject to immune complexome analysis (Fig. 1). Our observation supports that characteristic and substantial intrathecal humoral immune responses occur in infectious and CNS autoimmune diseases in which local B cells contribute to CSF autoantibody production [5]. Our method may be useful to *in vivo* screening the target antigens of oligoclonal band antibodies that are locally produced by clonally expanded antigen-experienced B cells [5] because the method is different from conventional *in vitro* antigen screening arrays using recombinant proteins. Most IC-associated antigens (140 of 176) in the CSF immune complexome were found to be exclusively detected by the immune complexome analysis. In the comparison between 140 antigens specific to the CSF immune complexome and previously-reported disease-associated CSF proteins, only a few (6 antigens) immune complexome-specific antigens were among the previously-reported disease-associated CSF proteins. This indicated that protein upregulation in CSF rarely leads to immunological responses producing ICs, and the other factors, such as mutation and misfold, may contribute to such responses. These findings also indicated that immune complexome analysis identified a group of proteins that was distinct from screening methods for disease-associated proteins in CSF. This is because selective enrichment for ICs increases sensitivity for IC-derived antigens that would otherwise be masked by whole CSF proteome. The specificity indicated that our method explores a new path for discovery of disease-specific or pathogen-specific markers in CSF.

Epidemiological studies during the past five decades demonstrate increasing prevalence of neuropsychiatric damage in patients with SLE and that this damage has a negative impact on survival [44]. Although there is considerable variation in the reported frequency of NPSLE, recent data from large cohorts indicate prevalence rates of approximately 30-40% [45]. Antinuclear and associated antibodies are widely used for diagnosis of SLE; however, the lowest prevalence of these markers is among patients with NPSLE, and the serological diagnosis of NPSLE is difficult [46]. The sensitivity of IC-associated SBSN for NPSLE was only 35% in the immune complexome analysis of CSF. However, our method is a screening method for discovering disease-associated immune complex antigens. The sensitivity of individual disease-specific antigens may be improved by developing ELISA methods that each detects a certain IC with high specificity and sensitivity [47]; if such ELISAs were developed, these disease-specific antigens may become promising diagnostic or pathogenic biomarkers. On the other hand, immune complexome analysis of diseased tissue is more straightforward to understand the antigenicity. However, it is difficult to recover the tissue from CNS; therefore, immune complexome analysis of patient's CSF is useful to screening the CNS autoimmune disease-specific IC antigens. Furthermore, if the epitope peptides of the antigens were identified, we can expand the study to develop an epitope-targeted therapy, which concludes the clinical and therapeutic benefit of the antigen.

SBSN was initially identified as an epidermal differentiation marker [48]; it was then reported to be epigenetically depressed in lung cancer [49, 50]. Our follow-up experiments showed that an anti-SBSN antibody was highly expressed in NPSLE patients

compared to SLE and MS, and induced interleukin-6 production with lipopolysaccharide stimulation in astrocytes [51]. Additionally, microarray data showed that the senescence, autophagy pathways and TGF-β signaling were significantly changed in astrocytes exposed to anti-SBSN antibody compared to normal immunoglobulin G exposure. Also, SBSN was reported not to be expressed in the brain of newborn mice [48]. These findings indicate that anti-SBSN antibody and its immune complex may be important to the pathogenesis of NPSLE [51]. These data illustrate the value and advantage of immune complexome analysis for identifying disease-associated IC antigens in CSF. The specific detection of SBSN in NPSLE patients was a clear example of the successful application of CSF immune complexome analysis.

Of the CNS autoimmune diseases investigated in this study, only NMO has been associated with a specific biomarker; reportedly, an autoantibody (anti-aquaporin-4 antibody) can distinguish NMO from the other three diseases, and especially from MS [52, 53]. Although all the NMO patients enrolled in this study were positive for anti-aquaporin-4 antibody, immune complexome analysis did not identify aquaporin-4 as an IC-associated antigen in CSF; IC-associated aquaporin-4 may be absent from CSF samples because aquaporin-4 is anchored within astrocyte membranes. Aquaporin-4 is anchored to astrocyte foot process membranes by the dystroglycan complex, and it faces the abluminal surface of blood vessels; moreover, aquaporin-4-antibody binding is followed by complement activation [54].

General proteomic approaches used for diagnostic or pathogenic biomarkers

discovery both identify and quantify proteins; however, there are often too many proteins that are differentially expressed between disease and control groups to be validated as biomarkers. In fact, these general approaches have delivered few if any useful diagnostic or pathogenic biomarker to clinical setting. In contrast, our method found the antigens that were detected only in a disease group and not in a control group. Therefore, naturally only a few antigens would be examined in subsequent validation studies. Our method includes the risk that some useful biomarkers are missed because they are detected in both groups and only expression levels differ between groups. However, the antigens specifically detected in a disease group are thought to be more promising candidates for biomarkers. Based on these observations, we suggest that immune complexome analysis is a promising approach to screening for diagnostic or pathogenic IC biomarkers in CSF.

Conclusions

This report is the first to comprehensively identify the antigens incorporated into ICs in CSF. This group of antigens has limited overlap with the CSF proteome or the plasma proteome; therefore, our method, which focused on ICs, explores a new avenue for discovery of CSF biomarkers that could be used for diagnosis, pathology, or both. Based on our analysis of CSF samples from cases of CNS autoimmune diseases, IC-associated SBSN was apparently specifically and frequently detected in CSF from patients with NPSLE. Further analyses involving a large number of NPSLE patients and a method specifically detecting SBSN-associated ICs are warranted to determine the clinical benefit of using IC-associated

SBSN as a biomarker.

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References

[1] B. Obermeier, R. Daneman, R. Ransohoff, R., Development, maintenance and disruption of the blood-brain barrier, Nat. Med. 19 (2013) 1584-96.

[2] H. Wolburg, K. Wolburg-Buchholz, B. Engelhardt, Diapedesis of mononuclear cells across cerebral venules during experimental autoimmune encephalomyelitis leaves tight junctions intact, Acta Neuropathol. 109 (2005) 181-90.

[3] L.M. Dallasta, L.A. Pisarov, J.E. Esplen et al, Blood-brain barrier tight junction disruption in human immunodeficiency virus-1 encephalitis, Am. J. Pathol. 155 (1999) 1915-27.

[4] B. Obermeier, R. Mentele, J. Malotka et al, Matching of oligoclonal immunoglobulin transcriptomes and proteomes of cerebrospinal fluid in multiple sclerosis, Nat. Med. 14 (2008) 688-93.

[5] S.M. Brändle, B. Obermeier, M. Senel et al, Distinct oligoclonal band antibodies in multiple sclerosis recognize ubiquitous self-proteins, Proc. Natl. Acad. Sci. USA 113 (2016) 7864-7869.

[6] C.P. Genain, B. Cannella, S.L. Hauser, C.S. Raine, Identification of autoantibodies associated with myelin damage in multiple sclerosis, Nat. Med. 52 (1999) 170-5.

[7] S.L. Hauser, E. Waubant, D.L. Arnold et al, B-cell depletion with rituximab in relapsing-remitting multiple sclerosis, N. Eng. J. Med. 358 (2008) 676-88.

[8] B.G. Weinshenker, P.C. O'Brien, T.M. Petterson et al, A randomized trial of plasma exchange in acute central nervous system inflammatory demyelinating disease, Ann. Neurol. 46 (1999) 878-86.

363 [9] A.C. Flach, T. Litk, J. Strauss et al, Autoantibody-boosted T-cell reactivation in the target
364 organ triggers manifestation of auoimmune CNS disease, Proc. Natl. Acad. Sci. USA 113
365 (2016) 3323-8.
366 [10] L. Klotz, I. Kuzmanov, S. Hucke et al, B7-H1 shapes T-cell-mediated brain endothelial
367 cell dysfunction and regional encephalitogenicity in spontaneous CNS autoimmunity,
368 Proc. Natl. Acad. Sci. USA 113 (2016) 6182-6191.
369 [11] G. Krishnamoorthy, H. Lassmann, H. Wekerle, A. Holz, Spontaneous opticospinal
370 encephalomyelitis in a double-transgenic mouse model of autoimmune T cell/B cell
371 cooperation, J. Clin. Invest. 116 (2006) 2385-2392.
372 [12] T. Berger, S. Weerth, K. Kojima, C. Linington, H. Wekerle, H. Lassmann, Experimental
373 autoimmune encephalomyelitis: the antigen specificity of T lymphocytes determines the
374 topography of lesions in the central and peripheral nervous system, Lab. Invest. 76 (1997)
375 355-364.
376 [13] F. Shimidzu, K.L. Schaller, G.P. Owens et al, Glucose-regulated protein 78 autoantibody
377 associates with blood-brain barrier disruption in neuromyelitis optica, Sci. Transl. Med. 9
378 (2017) eaai9111.
379 [14] J.R. Seibold, R.B. Buckingham, T.A. Medsger, R.H. Kelly, Cerebrospinal fluid immune
380 complexes in systemic lupus erythematosus involving the central nervous system, Semin.
381 Arthritis. Rheum. 12 (1982) 68-76.
382 [15] H. Jans, A. Heltberg, I. Zeeberg, J.H. Kristensen, T. Fog, N.E. Raun, Immune complexes
383 and the complement factors C4 and C3 in cerebrospinal fluid and serum from patients

with chronic progressive multiple sclerosis, Acta Neurol. Scand. 69 (1984) 34-8.

[16] P.K. Coyle, Detection and isolation of immune complexes in multiple sclerosis cerebrospinal fluid, J. Neuroimmunol 15 (1987) 97-107.

[17] R.A. Rudick, J.M. Bidlack, D.W. Knutson, Cerebrospinal fluid immune complexes that bind C1q, Arch. Neurol. 42 (1985) 856-8.

[18] F. Wiederkehr, M. Wacker, D. Vonderschmitt, Analysis of immune complexes of cerebrospinal fluid by two-dimensional gel electrophoresis, Electrophoresis 10 (1989) 473-9.

[19] P.K. Coyle, S.E. Schutzer, A.L. Belman, L.B. Krupp, M.G. Golightly, Cerebrospinal fluid immune complexes in patients exposed Borrelia burgdorferi: detection of Borrelia-specific and -nonspecific complexes, Ann. Neurol. 28 (1990) 739-744.

[20] T.C.A. Ferrari, L.C. Faria, T.S. Vilaça, C.R. Correa, A.M. Góes, Identification and characterization of immune complexes in the cerebrospinal fluid of patients with spinal cord schistosomiasis, J. Neuroimmunol. 230 (2011) 188-190.

[21] R.M. Ransohoff, B. Engelhardt, The anatomical and cellular basis of immune surveillance in central nervous system, Nat. Rev. Immunol. 12 (2012) 623-635.

[22] K. Ohyama, Y. Ueki, A. Kawakami et al, Immune complexome analysis of serum and its application in screening for immune complex antigens in rheumatoid arthritis, Clin. Chem. 57 (2011) 905-9.

[23] K. Ohyama, A. Kawakami, M. Tamai, M. Baba, N. Kishikawa, N. Kuroda, Serum immune complex containing thrombospondin-1: a novel biomarker for early rheumatoid

arthritis, Ann. Rheum. Dis. 71 (2012) 1916-7.

[24] K. Ohyama, M. Baba, M. Tamai et al, Proteomic profiling of antigens in circulating immune complexes associated with each of seven autoimmune diseases, Clin. Biochem. 48 (2015) 181-185.

[25] K. Ohyama, M. Baba, M. Yamamoto et al, Immune complexome analysis of antigens in circulating immune complexes isolated from patients with IgG4-related dacryoadenitis and/or sialadenitis, Mod. Rheumatol. 26 (2016) 248-250.

[26] N.T. Huy, H.T. Trieu, K. Okamoto et al, Proteomic profile of circulating immune complexes in dengue infected patients, J. Trop. Dis. 1 (2013) 109.

[27] K. Ohyama, N.T. Huy, H. Yoshimi et al, Proteomic profile of circulating immune complexes in chronic Chagas disease, Parasite Immunol. 38 (2016) 609-17.

[28] K. Ohyama, H. Yoshimi, N. Aibara et al, Immune complexome analysis reveals specific and frequent presence of immune complex antigens in lung cancer patients: A pilot study, Int. J. Cancer 140 (2017) 370-80.

[29] The American college of rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes, Arthritis Rheum. 42 (1999) 599-608.

[30] C.H. Polman, S.C. Reingold, B. Banwell et al, Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria, Ann. Neurol. 69 (2011) 292–302.

[31] D.M. Wingerchuck, V.A. Lennon, S.J. Pittock, C.F. Lucchinetti, B.G. Weinshenker, Revised diagnostic criteria for neuromyelitis optica, Neurology 66 (2006) 1485-9.

[32] S.E. Schutzer, T. Liu, B.H. Natelson et al, Establishing the proteome of normal human

cerebrospinal fluid, PLoS ONE 5 (2010) e10980.

[33] D.J. States, G.S. Omenn, T.W. Blackwell et al, Challenges in deriving high-confidence protein identification from data gathered by a HUPO plasma proteome collaborative study, Nat. Biotechnol. 24 (2006) 333-8.

[34] A.C. Kroksveen, J.A. Opsahl, T.T. Aye, R.J. Ulvik, F.S. Berven, Proteomics of human cerebrospinal fluid: Discovery and verification of biomarker candidates in neurodegenerative diseases using quantitative proteomics, J. Proteomics 74 (2011) 371-88.

[35] D. Chiasserini, J.R.T. van Weering, S.R. Piersma et al, Proteomic analysis of cerebrospinal fluid extracellular vesicles: A comprehensive dataset, J. Proteomics 106 (2014) 191-204.

[36] A.J. Percy, J. Yang, A.G. Chambers, R. Simon, D.B. Hardie, C.H. Borchers, Multiplexed MRM with internal standards for cerebrospinal fluid candidate protein biomarker quantitation, J. Proteome Res. 13 (2014) 3733-3747.

[37] Y. Zhang, Z. Guo, L. Zou et al, A comprehensive map and functional annotation of the normal human cerebrospinal fluid proteome, J. Proteomics 119 (2015) 90-99.

[38] A.N. Galindo, M. Kussmann, L. Dayon, Proteomics of cerebrospinal fluid: Throughput and robustness using a scalable automated analysis pipeline for biomarker discovery, Anal. Chem. 87 (2015) 10755-10761.

[39] A. Zougman, B. Pilch, A. Podtelejnikov et al, Integrated analysis of the cerebrospinal fluid peptidome and proteome, J. Proteome Res. 7 (2008) 386-99.

447 [40] M.P. Stoop, L.J. Dekker, M.K. Titulaer et al, Multiple sclerosis-related proteins identified
448 in cerebrospinal fluid by advanced mass spectrometry, Proteomics 8 (2008) 1576-85.
449 [41] F. Abdi, J.F. Quinn, J. Jankovic et al, Detection of biomarkers with a multiplex
450 quantitative proteomic platform in cerebrospinal fluid of patients with neurodegenerative
451 disorders, J. Alzheimers Dis. 9 (2006) 293-348.
452 [42] X. Yuan, D.M. Desiderio, Proteomics analysis of human cerebrospinal fluid, J.
453 Chromatogr. B 815 (2005) 179-89.
454 [43] A.F. Huhmer, R.G. Biringer, H. Amato, A.N. Fonteh, M.G. Harrington, Protein analysis in
455 human cerebrospinal fluid: Physiological aspects, current progress and future challenges,
456 Dis. Markers 22 (2006) 3-26.
457 [44] A. Mak, M.W. Cheung, H.J. Chiew et al, Global trend of survival and damage of
458 systemic lupus erythematosus: meta-analysis and meta-regression of observational
459 studies from the 1950 s to 2000 s, Semin. Arthritis Rheum. 41 (2012) 830–839.
460 [45] A.M. Borowoy, J.E. Pope, E. Silverman et al, Neuropsychiatric lupus: the prevalence and
461 autoantibody associations depend on the definition: results from the 1000 faces of lupus
462 cohort, Semin. Arthritis Rheum. 42 (2012) 179– 185.
463 [46] A.J. Swaak, V. Huysen, J.C. Nossent, R.J. Smeenk, Antinuclear antibody profiles in
464 relation to specific disease manifestations of systemic lupus erythematosus, Clin.
465 Rheumatol. 9 (Suppl 1) (1990) 82-94.
466 [47] J. Rho, P.D. Lampe, High-throughput screening for native autoantigen-autoantibody
467 complexes using antibody microarrays, J. Proteome Res. 12 (2013) 2311-20.

468 [48] G.T. Park, S.E. Lim, S.I. Jang, M.I. Morasso, Suprabasin, a Novel epidermal
469 differentiation marker and potential cornified envelope precursor. J. Biol. Chem. 47
470 (2002) 45195-202.
471 [49] C.A. Glazer, I.M. Smith, M.F. Ochs et al, Integrative discovery of epigenetically
472 derepressed cancer testis antigens in NSCLC, PLoS ONE 4 (2009) e8189.
473 [50] D. Gaykalova, R. Vatapalli, C.A. Glazer et al, Dose-dependent activation of putative
474 oncogene SBSN by BORIS, PLoS ONE 7 (2012) e40389.
475 [51 K. Ichinose, K. Ohyama, K. Furukawa et al, Novel anti-suprabasin antibodies may
476 contribute to the pathogenesis of neuropsychiatric systemic lupus erythematosus, Clin.
477 Immunol., https://doi.org/10.1016/j.clim.2017.11.006
478 [52] V.A. Lennon, D.M. Wingerchuk, T.J. Kryzer et al, A serum autoantibody marker of
479 neuromyelitis optica: distinction from multiple sclerosis, Lancet 364 (2004) 2106-2112.
480 [53] V.A. Lennon, T.J. Kryzer, S.J. Pittock, A.S. Verkman, S.R. Hinson IgG marker of
481 optic-spinal multiple sclerosis binds to the aquaporin-4 water channel, J. Exp. Med. 202
482 (2005) 473-477.
483 [54] S.F. Roemer, J.E. Parisi, V.A. Lennon et al, Pattern-specific loss of aquaporin-4
484 immunoreactivity distinguishes neuromyelitis optica from multiple sclerosis, Brain 130
485 (2007) 1194-1205.

Figure captions

Fig. 1 Total ion chromatograms of proteins isolated from (a) CSF or (b) serum via immune complexome analysis.

Fig. 2 A Venn diagram representing proteins that were identified as IC-associated antigens in CSF, constituents of the CSF proteome, constituents of the plasma proteome, or some combination thereof. The human CSF proteome dataset reported by Schutzer *et al.* [32] comprises 2628 proteins, and the HUPO plasma proteome dataset comprises 3020 proteins [33]; both were used as reference datasets.

Table 1 Disease-associated antigens that were detected in one or two disease groups.

Protein	Accession	NPSLE (n=26) Sensitivity (%)/Specificity (%)	NMO (n=16) Sensitivity (%)/Specificity (%)	MS (n=15) Sensitivity (%)/Specificity (%)	Hashimoto (n=7) Sensitivity (%)/Specificity (%)	AD (n=10) Sensitivity (%)/Specificity (%)
Isoform 7 of Nesprin-1	IPI00396977.2	8 / 100				
Isoform 10 of Fibronectin	IPI00479723.5		13 / 100			
Isoform 12 of Fibronectin	IPI00556632.4		13 / 100			
Isoform B of Fibulin-1	IPI00218803.3		13 / 100			
suprabasin isoform 1 precursor	IPI00947285.1	35 / 100				
Calmodulin-like protein 5	IPI00021536.2	12 / 92		27 / 95		
cDNA FLJ58075, highly similar to Ceruloplasmin	IPI00947307.1	4 / 96			29 / 99	
complement C4-B preproprotein	IPI00418163.3		13 / 100			
Desmoglein-1	IPI00025753.2	15 / 96		13 / 93		
Integrator complex subunit 4-like protein 2	IPI00102193.5	4 / 96				20 / 98
Isoform 1 of Alpha-1-antitrypsin	IPI00553177.1	8 / 98				10 / 97
Isoform 1 of Uncharacterized protein C9orf174	IPI00292836.4		6 / 97	13 / 98		
Isoform 2 of Golgi membrane protein 1	IPI00759659.1		6 / 97			20 / 98

Isoform 2 of Nuclear mitotic apparatus protein 1	IPI00006196.3	4 / 96		13 / 98		
Isoform 2 of Protein piccolo	IPI00789624.4	8 / 98			14 / 97	
Isoform 3 of Rapamycin-insensitive companion of mTOR	IPI00166528.4	19 / 100				
Isoform C of Fibulin-1	IPI00296537.4		31 / 100			
Putative uncharacterized protein DKFZp686G11190	IPI00784842.1		13 / 100			

Fig. 1

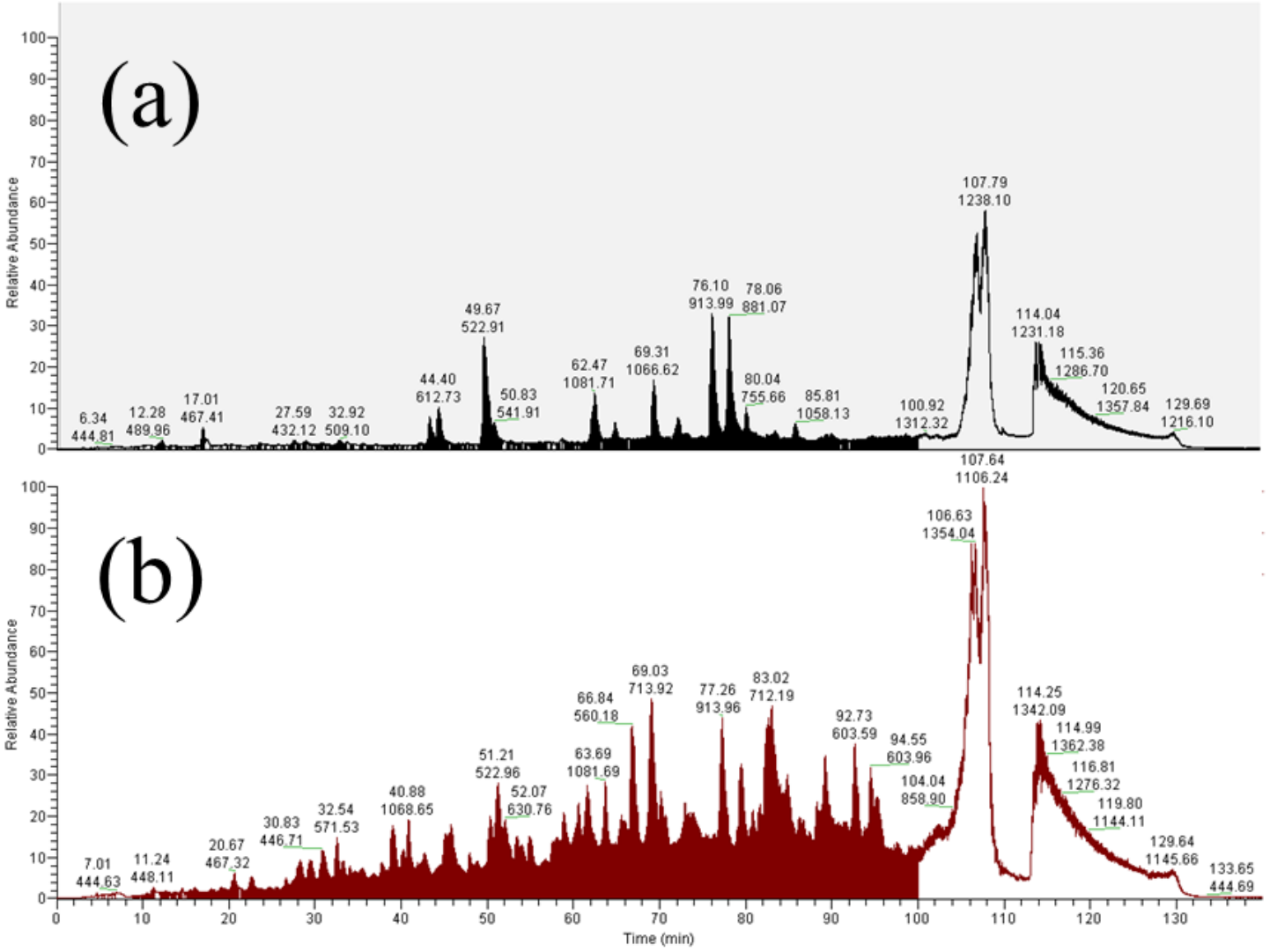
(a)
Relative Abundance
6.34 444.81
12.28 489.96
17.01 467.41
27.59 432.12
32.92 509.10
44.40 612.73
49.67 522.91
50.83 541.91
62.47 1081.71
69.31 1066.62
76.10 913.99
78.06 881.07
80.04 755.66
85.81 1058.13
100.92 1312.32
107.79 1238.10
114.04 1231.18
115.36 1286.70
120.65 1357.84
129.69 1216.10
(b)
Relative Abundance
7.01 444.63
11.24 448.11
20.67 467.32
30.83 446.71
32.54 571.53
40.88 1068.65
51.21 522.96
52.07 630.76
63.69 1081.69
66.84 560.18
69.03 713.92
77.26 913.96
83.02 712.19
92.73 603.59
94.55 603.96
104.04 858.90
106.63 1354.04
107.64 1106.24
114.25 1342.09
114.99 1362.38
116.81 1276.32
119.80 1144.11
129.64 1145.66
133.65 444.69
Time (min)

Fig. 2