Identification of Three New Autoantibodies Associated with Systemic Lupus Erythematosus Using Two Proteomic Approaches

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Our objective was to identify new serum autoantibodies associated with systemic lupus erythematosus (SLE), focusing on those found in patients with central nervous system (CNS) syndromes. Autoantigens in human brain proteins were screened by multiple proteomic analyses: two-dimensional polyacrylamide gel electrophoresis/Western blots followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis and immunoprecipitation followed by liquid chromatography-tandem mass spectrometry shotgun analysis. The presence of serum IgG autoantibodies against 11 selected recombinant antigens was assessed by Western blot and enzyme-linked immunosorbent assay (ELISA) in the sera of 106 SLE patients and 100 normal healthy controls. The O.D. values in sera from SLE patients were significantly higher than those of controls for the antigens crystallin αB (p = 0.0002), esterase D (p = 0.0002), APEX nuclease 1 (p < 0.0001), ribosomal protein P0 (p < 0.0001), and PA28γ (p = 0.0005); the first three are newly reported. The anti-esterase D antibody levels were significantly higher in the CNS group than in the non-CNS group (p = 0.016). Moreover, when the SLE patients were categorized using CNS manifestations indicating neurologic or psychiatric disorders, the anti-APEX nuclease 1 antibody levels were significantly elevated in SLE patients with psychiatric disorders (p = 0.037). In conclusion, the association of SLE with several new and previously reported autoantibodies has been demonstrated. Statistically significant associations between anti-esterase D antibodies and CNS syndromes as well as between anti-APEX nuclease 1 antibodies and psychiatric disorders in SLE were also demonstrated. The combined immunoproteomic approaches used in this study are reliable and effective methods for identifying SLE autoantigens. Molecular & Cellular Proteomics 10: 10.1074/mcp.M110.005330, 1–12, 2011.

Systemic lupus erythematosus (SLE) is an autoimmune disease that usually develops in women aged 18–50 years and is characterized by the presence of autoantibodies. Diagnosis is difficult because SLE is a great imitator of other diseases (1). Autoantibodies are clearly central to the pathogenesis of SLE, and different autoantibodies are associated with different clinical features (2). Several of the more than 100 autoantibodies identified to date have been associated with disease activity (1). Although anti-double-stranded DNA antibodies are the most extensively studied autoantibodies in SLE, others play roles in clinical manifestations, particularly in autoimmune hemolytic anemia, thrombocytopenia, skin disease, and neonatal lupus (3).

Central nervous system (CNS) lupus is a serious and potentially life-threatening manifestation of SLE, occurring in 37–95% of cases, and is associated with increased risk of death (4). Despite its frequency and severity, the lack of a diagnostic gold standard makes it challenging to differentiate primary CNS lupus from secondary neuropsychiatric (NP) manifestations unrelated to SLE at their onset (4–6). The American College of Rheumatology (ACR) has developed a standardized nomenclature system to provide case definiti...
Proteomic Identification of Novel Antibodies in SLE

The possibility of simultaneously measuring different autoantibodies in the sera of patients suffering from autoimmune diseases (8) has not been extensively studied, and they may be merely an epiphenomenon (7). The identification and characterization of new, specific autoantibodies could help elucidate the etiology of the NP manifestations that accompany SLE, opening new perspectives for more effective diagnostic and therapeutic strategies.

Conventional study of the autoimmune response has been conducted by analyzing the presence and/or concentration of individual antibodies in biological fluids. Proteomic techniques allow the simultaneous identification and measurement of different autoantibodies in the sera of patients suffering from autoimmune diseases (8). Recent advances in proteomic technologies have enabled large-scale profiling of proteins in tissues and sera from patients and provided an unprecedented ability to identify novel biosignatures useful in diagnosing and classifying autoimmune diseases and guiding therapeutic decision making in patients with these disorders, including SLE (9–15). The possibility of simultaneously measuring a number of correlated analytes is interesting for analytical reasons (e.g., reduced biological sample and reagent volumes and lower costs), logistical and managerial reasons, and pathophysiological reasons (i.e., identifying combinations of markers for use in disease-oriented or organ-oriented profiling) (8). However, much work remains unfinished in developing, refining, validating, and applying proteomics technologies to identify biomarkers in autoimmune diseases (9).

By using both conventional and newer proteomic approaches, our aim was to find novel serum autoantibodies associated with SLE, focusing on those found in patients with CNS syndromes.

**EXPERIMENTAL PROCEDURES**

**Study Subjects and Sample Collection**—Sera from 106 patients with active SLE from 1994 through 2007 were obtained using the Tokyo Women’s Medical University SLE Database. These sera were originally collected from the patients’ whole blood using standard tubes with a polyester gel separator. Immediately following clotting at room temperature and following centrifugation, the separated sera were aliquoted and stored at −80 °C. All patients had four or more revised ACR (formerly the American Rheumatism Association) criteria for SLE (16, 17) and gave informed consent for inclusion in this study. Those who had non-SLE-related NP manifestations arising from infection, uremia, electrolyte imbalance, hypoxia, brain tumor, trauma, primary mental disease, drug use or past histories of NP involvement were excluded. These patients were excluded because we wanted to compare recently diagnosed, active CNS lupus patients to non-NPSLE patients; unrelated conditions could affect current symptoms or laboratory findings. At the time of serum collection, each patient underwent a standardized medical history that included medication use and was given a physical examination that included neurologic and rheumatologic assessments. Psychiatric examinations were employed when indicated. Serology profiling for each patient was performed using standard immunoassays. Subjects were classified into the CNS group or the non-CNS group according to the presence or absence of active CNS syndromes. The CNS group was then further classified into the neurologic disorders group consisting of patients with neurologic disorders with or without other NP syndromes, or the psychiatric disorders group comprising patients with psychiatric disorders with or without other NP syndromes (5, 18, 19). Detailed diagnostic criteria for these groups are described below. Control sera were derived from age- and sex-matched healthy donor subjects and from patients with rheumatoid arthritis (RA), systemic sclerosis (SSc), Sjögren syndrome (SS), and multiple sclerosis (MS) diagnosed using standard criteria (20–23). This study was approved by the Ethical Committee of our institution and the Helsinki Declaration was followed throughout the study.

**Diagnosis of CNS Lupus**—Although ACR nomenclature and case definitions include 12 CNS syndromes and seven peripheral nervous system syndromes (5, 18, 19), we used only the 12 CNS syndromes in the inclusion criteria for our study because of the substantial differences between the central and peripheral nervous systems in anatomy, function, and clinical characteristics. Slight or mild cognitive dysfunction without significant clinical impairment, as revealed by detailed neuropsychological testing, was excluded from the CNS syndromes in our study. Tension headache and episodic tension type headache were also excluded. CNS syndromes were further classified into neurologic disorders (aseptic meningitis, cerebrovascular disease, demyelinating syndrome, headache, movement disorder, myelopathy, and seizure disorders) and psychiatric disorders (acute confusional state, anxiety disorder, cognitive dysfunction, mood disorder, and psychosis) (5).

The final clinical diagnosis and classification of the various NP syndromes for inclusion in the study were made by an experienced rheumatologist (M. H.) and psychiatrist (K. N.), according to the standardized ACR nomenclature and case definitions for NP lupus syndromes (5). These decisions were based on the medical history and neuropsychological examinations by rheumatologists, an experienced neurologist (S. U.) and a psychiatrist (K. N.) and were supported by conventional laboratory tests and appropriate complementary tests, including MRI, electroencephalography, and cerebral spinal fluid tests, as well as an assessment of the clinical course of the disease.

**Cell Culture**—Human neuroblastoma cell lines (IMR-32 and NB-1) and human glioblastoma cell lines (A172 and T98G) were obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). IMR-32 cells were routinely cultured in MEM (Sigma-Aldrich, St. Louis, MO) supplemented with nonessential amino acids (MP Biomedicals, Irvine, CA). NB-1 cells were cultured in a medium containing an equal amount of MEM and RPMI 1640 (Sigma-Aldrich). The human glioblastoma cell lines, A172 and T98G, were cultured in RPMI 1640. All culture media were supplemented with 10% fetal bovine serum (SAFC Biosciences, Lenexa, KS), 50 U/ml of penicillin, and 50 μg/ml of streptomycin.

**Protein Preparation for Screening of Candidate Autoantigens**—The total protein from human whole brain (BioChain Institute, Hayward, CA) was precipitated once using the ReadyPrep 2-D Cleanup Kit (Bio-Rad Laboratories, Hercules, CA), according to the manufacturer’s protocol and used for two-dimensional polyacrylamide gel electrophoresis. The cultured cells were washed with phosphate buffered saline (PBS), scraped into a 1.5-ml tube and centrifuged to harvest.
cells. To prepare total cell protein, the cell pellet was sonicated on ice [output: 2; duty: 60; for 1 min (min); Sonifuer 250D; Branson Ultrasonics Corporation, Danbury, CT] in 6 M urea, 2 M thiourea, 4% 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid (CHAPS), and a complete EDTA-free protease inhibitor mixture (Roche Diagnostics GmbH, Mannheim, Germany). Following centrifugation at 20,400 × g for 10–30 min at 4 °C, the supernatant was recovered as total cell protein for use in the screening of autoantigens. Similarly, as for total protein from human whole brain, the total cell protein was precipitated using the ReadyPrep 2-D Cleanup Kit. Protein concentrations were measured using the bicinchoninic acid protein assay reagent (Pierce, Rockford, IL) according to the manufacturer’s protocol, using bovine serum albumin (BSA) as the standard.

Two-dimensional Polyacrylamide Gel Electrophoresis (Two-dimen-
sional-PAGE)—Human brain proteins were screened for autoantigens using two proteomic analysis techniques. First, we performed two-dimen-
sional-PAGE/Western blots (WBs) and then analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF/MS). Two-dimensional-PAGE was performed as de-
scribed elsewhere (24). Briefly, prepared protein samples were pre-
cipitated using the ReadyPrep 2-D Cleanup Kit (Bio-Rad) according to the manufacturer’s protocol. Before electrophoresis, the protein pel-
lets were dissolved in DeStreak Rehydration Solution (GE Healthcare, Buckinghamshire, UK) containing 0.2% BioLyte 3/10 (Bio-Rad) and 50 mM dithiothreitol. Next, 50 µg of protein was applied to IPG ReadyStrip (pH 3–10, nonlinear, 7 cm long; Bio-Rad) for overnight in-gel rehydration. The proteins were then separated by one-dimen-
sional isoelectric focusing (Multiphore II (GE Healthcare) or CoolPhor-
eSter IPG-IEF Type-P (Anatech, Tokyo, Japan) at 20 °C and focused with the following program: 200 V for 18 min, 500 V for 18 min, 1000 V for 18 min, 1500 V for 18 min, 2000 V for 18 min, 2500 V for 18 min, 3000 V for 18 min, and 3500 V for 90 min. Following one-dimensional isoelectric focusing, the IPG strips were equilibrated in a solution of 50 mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS, and 0.002% bromphenol blue that also contained 10 mg/ml dithiothreitol for the first equilibration step and 25 mg/ml iodoacetamide in the second equilibration step; the strips were bathed in solution on an orbital shaker at room temperature for 15 min in each step. The equilibrated strips were then sealed with 1% SeaKem GTG Agarose (Lonza, Rockland, ME) in SDS running buffer at the top of two-
dimensional SDS-polyacrylamide gels. SDS-PAGE was performed using precast 5–20% polyacrylamide gradient gels (SuperSea-
P 5–20%, two-dimensional gel; Wako Pure Chemical Industries, Osaka, Japan) at a constant current of 20 mA/gel until the bromphenol blue dye front reached the lower edge of the gel.

WB Analysis of Two-dimensional-PAGE Using Patient Sera—Follow-
ning two-dimensional-PAGE, the separated proteins were trans-
ferred onto Immobilon-P (Millipore, Billerica, MA) with a semidry blot-
ning technique. After blocking with 5% nonfat dry milk in PBS-T (PBS containing 0.05% Tween 20 in PBST), the immunoblot membranes were labeled using Cy5 Mono-Reactive Dye (GE Healthcare) for 30 min at room temperature. A 1:500 dilution of serum mixtures from nine SLE patients was used. The membranes were blocked for 1 h at room temperature with blocking buffer (1% skim milk in PBS-T). WBs were performed using the pooled sera from SLE patients with active CNS syndromes (the CNS group) or those without either current CNS syndromes or a history of CNS syndromes (the non-CNS group) diluted 1:500 in blocking buffer and incubated overnight at 4 °C. Following being washed with PBS-T, the membranes were incubated for 1 h at room temperature with the secondary antibody goat anti-human IgG (H+L) (Zymed Laboratories Inc., San Francisco, CA), conjugated to horseradish peroxidase (HRP), and diluted 1:15,000 in blocking buffer. WBs were visualized using the ECL plus (GE Healthcare) chemiluminescence signal, and proteins on the membrane were visualized using the Cy5 fluorescence signal. These fluorescent images were scanned using a Typhoon 9400 (GE Healthcare) with excitation at 457 nm and emission filter 520BP40 (ECL plus) and with excitation at 633 nm and emission filter 670BP30 (Cy5). The resulting images were overlaid and easily matched to identical positions from the western signal and protein signal. Using this information, protein samples were prepared for mass spectrom-
etry analysis.

MALDI-TOF/MS—Using the results of the two-dimensional-PAGE/ WB, the ECL plus signal spots specific for CNS lupus patients’ pooled sera were detected, and their positions were identified on the Cy5-
protein signals. For MALDI TOF/MS analysis, the proteins were sep-
carated using two-dimensional-PAGE, transferred onto a ProBlott membrane (Applied Biosystems, Foster City, CA) and stained with Coomassie Brilliant Blue R-250 (PhastGel Blue R, GE Healthcare). CNS lupus-specific spots were excised and washed with Milli-Q water. The isolated proteins were then digested at 37 °C for 90 min using 1 pmol/µl of lysine endopeptidase (mass spectrometry grade, Wako Pure Chemical Industries) in 50% acetonitrile. The resulting peptides were purified using NuTip NT11HL 96 solid-phase extraction cartridges (Glyken, Columbia, MD) and mixed with α-cyano-4-hy-
droxycinnamic acid matrix. Peptide mass fingerprinting was per-
formed using a MALDI-TOF mass spectrometer ( Voyager™, Applied Biosystems). Peptide mass fingerprinting data were compared with the NCBI n databases (human, 233173 sequences; date 2010/12/24) using the Mascot Search engine (Peptide Mass Fingerprint, version 2.2; Matrix Science, Boston, MA) with the following parameters: En-
zymes, Lys-C; Variable modifications, Carbamidomethyl (C) and Oxi-
dation (M); Mass values, Monoisotopic; Protein Mass, Unrestricted; Peptide Mass Tolerance: ± 0.2–0.4 Da; Peptide Charge State, 1+; and Max Missed Cleavages, 0 and exceeded the thresholds (p < 0.05). Confidence in the reliability of the identification was indicated by the number of matching and total peptides as well as the protein sequence coverage by the matching peptides.

Preparation of Antigens for Immunoprecipitation—Because two-
dimensional-PAGE/WB and MALDI TOF/MS analyses did not have sufficient sensitivity, we also used immunoprecipitation and protein shotgun analyses using liquid chromatography-tandem mass spec-
 trometry (LC-MS/MS) to screen candidate autoantigens. In the first trial, four types of cell lines (NB-1, IMR-32, A152, and T98G) were cul-
tured, washed with PBS, and harvested. Cell pellets were sus-
 pended in a cell lysis buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 2 mM MgCl2, 10 mM NaF, 2 mM EDTA, 25 mM β-glycerophosphate, 2 mM dithiothreitol, 1% Nonidet P-40, 10% glycerol, and 0.2% benzo-
nase nuclease [Novagen, Darmstadt, Germany]) containing a prote-
age inhibitor mixture (Roche, Basel, Switzerland) and used to prepare cell lysate for each of the four cell lines was mixed. In a separate assay, 10 mg of total protein from human whole brain (BioChain Institute) was added to this mixture of lysates from the four types of cell lines (5 mg each). These antigen mixtures were preclayed by incubation with 150 µl Protein G Sepharose beads (GE Healthcare) to prevent nonspecific proteins from binding to the beads and then used as antigens for immunoprecipitation.

Preparation of Total IgG Immobilized by Protein G Sepharose Beads—Total IgG from 67.5 µl of serum mixtures from nine SLE patients with active CNS syndromes and from nine SLE patients without active CNS syndromes was incubated with 37.5 µl of Protein G-Sepharose beads at room temperature using a gentle rocking motion for 45 min. The beads were then centrifuged and the super-

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natant was removed. The beads were washed twice with 0.2 M borate buffer (pH 9.0) and the IgG was crosslinked to the Protein G Sepharose beads by incubation at room temperature with 5 mM disuccinimidyl suberate (Pierce) for 30 min. The beads were washed with 0.2 M glycine (pH 8.0) and again incubated at room temperature for 30 min to block the active sites of disuccinimidyl suberate. The beads were then equilibrated with cell lysis buffer.

**Immunoprecipitation of Autoantigens and Total IgG—**A total of 7.5 mg of the antigens prepared as described above was added to the IgG immobilized beads and incubated at 4 °C for 90 min with agitation. Following centrifugation, the pellets were washed with washing buffer (20 mM HEPES, pH 7.5, 650 mM NaCl, 2 mM MgCl₂, 10 mM NaF, 2 mM EGTA, 25 mM β-glycerophosphate, 1% Nonidet-P-40, and 10% glycerol) three times. The IgG bound proteins were then eluted with 0.1 M glycine-HCl, pH 2.4, and neutralized with 2 M Tris-HCl, pH 8.0, 1.5 M NaCl, and 1 mM EDTA. The autoantigen proteins thus obtained were desalted and concentrated by methanol/chloroform precipitation (25–27).

**Sample Preparation for LC-MS/MS Analysis—**The samples were prepared for LC-MS/MS analyses as previously described (28). Briefly, the autoantigen pellet was dissolved in 100 μl of 1.2 M urea and 80 mM Tris-HCl, pH 8.5. The autoantigen was digested with trypsin (250 ng added directly to the solution at 37 °C for 16 h, Trypsin Sequencing Grade, modified; Roche), desalted using a ZipTip (C18; Millipore), and finally concentrated to approximately 20 μl to inject into the nanoflow system.

**Protein Shotgun Analysis by LC-MS/MS—**Protein shotgun analyses were performed using LC-MS/MS as previously described (28). Briefly, following the peptide mixture was applied to a C18 column (800-μm-inner-diameter and 3-mm-long), reverse-phase separation of the captured peptides was performed using a column (150-μm-inner-diameter and 75-mm-long) filled with HiQ-Sil C18 (3-μm particles, 120-A pores; KYA Technologies, Tokyo, Japan) with a direct nanoflow LC system (Dina, KYA Technologies). The peptides were eluted with a linear 5–65% gradient of acetonitrile containing 0.1% formic acid over 120 min at a flow rate of 200 nl/min and sprayed into a quadrupole time-of-flight tandem mass spectrometer (Q-ToF™ 2, Micromass/Waters, Milford, MA) (29). The MS/MS spectra were acquired using the following parameters: dynamic exclusion time, 120 s; duty cycle, 2 s; and mass tolerance, 0.1 Da. The MS/MS signals were then converted to text files by MassLynx (version 3.5, Micromass/Waters) and processed against the NCBI RefSeq human protein database (38963 sequences as of July 5, 2010) using the Mascot algorithm (MS/MS Ion Search, version 2.2.04, Matrix Science) with the following parameters: variable modifications, Acetyl (Protein N-term), Gin->pyro-Glu (N-term Q), and Oxidation (M); maximum missed cleavages, 2; peptide mass tolerance, 200 ppm; and MS/MS tolerance, 0.5 Da. The criterion for protein identification was based on having at least one MS1/MS data with Mascot scores that exceeded the thresholds (p < 0.05). A randomized decoy database created by the thresholds because of sample and antigen availability.

**Statistical Analyses—**The results of the ELISAs were correlated with the final clinical diagnosis for each patient. Two-group comparisons were analyzed using the Mann-Whitney U test for continuous variables. The Steel multiple comparison test was applied when appropriate. Values of p < 0.05 were considered statistically significant. Sensitivity, specificity, positive predictive value, and negative predictive value for the diagnosis were also calculated for some ELISAs. All statistical analyses were performed using JMP statistical software (version 7.0; SAS Institute, Cary, NC).

**RESULTS**

**Clinical Characteristics of SLE Patients—**Of the 106 patients with SLE enrolled in the present study, 100 were women and six were men. The median age of the patients was 31 years (range from 16 to 68 years). The median disease duration since diagnosis of SLE was 1 year (with a range of 0 to 20 years). The patients were all Japanese, except for 1 woman who was Chinese. Recently (i.e. within

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the last month) diagnosed, active CNS syndromes were observed in 32 patients (the CNS group), whereas the remaining 74 patients had neither current CNS syndromes nor a history of CNS syndromes (the non-CNS group). A neuropsychiatric disorder was diagnosed in 21 subjects, a psychiatric disorder was diagnosed in 15 subjects, and both disorders were diagnosed in four subjects. The final clinical diagnoses and classifications of the various NP syndromes for inclusion in the study were made according to the standardized ACR nomenclature and case definitions for NP lupus syndromes (5, 18, 19). There were no significant differences in other clinical parameters such as sex, age, or disease duration between the CNS group and the non-CNS group (p = 0.78, 0.78, and 0.68, respectively).

**Autoantigen Screening by Two-dimensional-PAGE and WB**—Autoantigens were screened for this study using two methods of proteomic analysis. First, we performed two-dimensional-PAGE/WB and MALDI TOF/MS analysis, a rather conventional approach for proteomic analysis (9). Total protein obtained from human whole brain or cell lysates of human neuroblastoma cell lines IMR-32 and NB-1 or human glioblastoma cell lines A172 and T98G was prepared as described in the Experimental Procedures and separated using two-dimensional-PAGE. Following two-dimensional-PAGE, the separated proteins were transferred onto membranes. Before WB, the proteins on the membrane were labeled with Cy5 Mono-Reactive Dye to allow the determination of their positions. The WB procedure was performed using pooled sera from SLE patients with active CNS syndromes (the CNS group) or those without either current CNS syndromes or a history of CNS syndromes (the non-CNS group), and proteins were visualized using ECL plus chemifluorescence. The resulting images were overlaid and easily matched to identical positions based on the western signal and protein-conjugated dye signal (Fig. 1A and 1B). Using this information, protein samples were prepared for mass spectrometry.

**Identification of Proteins by MALDI-TOF/MS and Mascot Search (Peptide Mass Fingerprint)**—Differentially reacted protein spots specific to the CNS group were excised from the blotted membranes and subjected to digestion with lysine endopeptidase, followed by peptide-mass fingerprinting and MALDI-TOF/MS. The peptide mass fingerprints obtained were used to search the NCBI database using the Mascot Search engine. Twelve protein spots from whole human brain total protein and four from human tumor cell line total protein were significantly and exclusively detected with pooled sera from the CNS group (circled in Fig. 1A and 1B, respectively, and summarized in Table I). Although many spots were found by this method, most of them were difficult to identify because of their low expression levels; too many minor proteins were present in each spot for the sensitivity of this method.

**Autoantigen Screening by Immunoprecipitation of Human Brain Proteins with SLE Patient Total IgG**—Two-dimensional-PAGE is labor intensive and often has the disadvantages of poor reproducibility, lack of sensitivity, and low throughput. Several newer approaches have recently been introduced to address some of these limitations in quantitative serum proteome analysis (9). Because two-dimensional-PAGE/WB followed by MALDI-TOF/MS (two-dimensional-PAGE/WB/MALDI-TOF/MS) analysis did not have sufficient sensitivity for our study, we explored a new approach utilizing immunoprecipitation followed by LC-MS/MS shotgun analysis. Total protein from human whole brain and human brain tumor cell lines was prepared as described above in the Experimental Procedures. Total IgG from serum mixtures from SLE patients with active CNS syndromes (the CNS group) or those without either current CNS syndromes or a history of CNS syndromes (the non-CNS group) was bound to Protein G-Sepharose beads. Prepared antigens were immunoprecipitated using the patients’ total IgG immobilized onto Protein G Sepharose beads.

**Identification of the Proteins by LC-MS/MS Shotgun Analysis and Mascot Search (MS/MS Ion Search)**—The autoantigen protein pellets were digested using trypsin, and the peptide fragments were analyzed on a nanoflow LC-MS/MS system. The LC-MS/MS shotgun analyses were performed twice for each sample group: the CNS and the non-CNS SLE groups. Proteins were identified from the MS/MS spectra and the NCBI database using the Mascot Search engine (MS/MS Ion Search). A total of 154 proteins, most not found using the two-dimensional-PAGE/WB/MALDI-TOF/MS analysis, were identified using this method (as shown in the supplemental data). Among these proteins, 50 were identified in the CNS group, but not in the non-CNS group (summarized in Table II).

**WB analysis of Serum IgG Autoantibodies Reactivities Against Discriminant Recombinant Antigens**—Although some of the proteomic data (e.g. esterase D) were insufficient for protein identification by themselves (supplemental data), we selected possible antigens based on not only the results of
Table I

| Source     | Spot No. | Protein name                                      | Accession No. | Coverage (%) | Protein score |
|------------|----------|--------------------------------------------------|---------------|--------------|---------------|
| Whole brain| 1        | Vesicle-fusing ATPase                             | NP_006169     | 18           | 106           |
|            | 2        | Succinate dehydrogenase flavoprotein subunit,    | NP_004159     | 12           | 68            |
|            |          | mitochondrial                                     |               |              |               |
|            | 3        | Septin 11                                        | NP_060713     | 22           | 111           |
|            | 4        | Isocitrate dehydrogenase subunit alpha,          | NP_005521     | 16           | 67            |
|            |          | mitochondrial                                     |               |              |               |
|            | 5        | Glutamate-ammonia ligase                          | NP_002056     | 21           | 102           |
|            | 6        | Fructose bisphosphate aldolase C                  | NP_005156     | 26           | 123           |
|            | 7        | Phosphoglycerate kinase 1/Cell migration-inducing| NP_000282     | 22           | 74            |
|            |          | gene 10 protein                                   |               |              |               |
|            | 8        | Aldolase A                                        | NP_000025     | 16           | 83            |
|            | 9        | Esterase D/S-formylglutathione hydrolase          | NP_001975     | 41           | 67            |
|            | 10       | ES1 protein homolog, mitochondrial                | NP_004640     | 25           | 70            |
|            | 11       | Phosphatidylethanolamine-binding protein 1        | NP_002558     | 72           | 163           |
|            | 12       | Crystallin αB/crystallin B chain                  | NP_001876     | 37           | 74            |
| Cell line  | 13       | Heterogeneous nuclear ribonucleoprotein K         | NP_002131     | 21           | 69            |
|            | 14       | 60S acidic ribosomal protein P0                    | NP_000993     | 34           | 98            |
|            | 15       | Triphosphate isomerase                            | AAH07086      | 51           | 156           |
|            | 16       | GTP-binding nuclear protein Ran                   | NP_006316     | 33           | 75            |

The two proteomic approaches described above also revealed new information. Among the many candidate antigens screened, 11 recombinant antigens showed positive reactions against the pooled sera of 28 patients with active CNS lupus by WB analysis: crystallin αB, esterase D, APEX nuclease 1, 60S acidic ribosomal protein P0 (ribosomal protein P0), proteasome activator complex subunit 3 (PA28γ), triosephosphate isomerase, phosphoglycerate kinase 1 (cell migration-inducing gene 10 protein), phosphatidylethanolamine-binding protein 1, phosphoglycerate mutase 1, 3-hydroxyacyl-CoA dehydrogenase type-2, and glutaric acid dehydrogenase (Fig. 2). These were included in those for patients with the other diseases and NHCs for APEX nuclease 1 using sera from patients with RA, SSc, SS, and MS; esterase D was not studied because of the limited volumes of antigen available. The ELISA-generated O.D. values for sera from SLE patients were significantly higher than those for patients with the other diseases and NHCs for APEX nuclease 1 (SLE versus all other controls using the Steel multiple comparison test; \( p < 0.0001 \)), but not significantly different for crystallin αB (see Fig. 4A and 4B, respectively). Using a theoretical cutoff titer of 0.39 (O.D.) for anti-APEX nuclease 1 antibodies as the NHC mean + 3SD, their prevalence in patients with SLE, RA, SSc, SS, and MS and NHCs was 20%, 13%, 8%, 5%, 5%, and 2%, respectively. When samples of SLE patients and NHCs were compared, the sensitivity, specificity, positive predictive value, and negative predictive value of the anti-APEX nuclease 1 antibodies for the diagnosis of SLE were 20%, 98%, 95%, and 33%, respectively.

Comparisons of the SLE-related Autoantibody Titers Among the CNS Group and the non-CNS Group Using ELISA—The O.D. values of sera from the CNS group were significantly higher than those from the non-CNS group in ELISAs using esterase D (\( p = 0.016 \)), although most of them show low O.D. values (< 0.1; Fig. 5A). Unexpectedly, no O.D. values in ELISAs of other autoantibodies differed significantly between SLE patients with or without active CNS syndromes when tested in larger sample sizes: crystallin αB (\( p = 0.75 \)).
APEX nuclease 1 (p = 0.18), ribosomal protein P0 (p = 0.89), or PA28γ (p = 0.58). However, when the CNS group with neurologic disorders and the CNS group with psychiatric disorders were separately compared with SLE patients without those disorders, the levels of anti-APEX nuclease 1 antibodies were significantly elevated in SLE patients with psychiatric disorders compared with the levels in SLE patients without psychiatric disorders (p = 0.037; Fig. 5B).

### TABLE II
Identified CNS lupus-specific proteins by immunoprecipitation followed by LC-MS/MS shotgun analysis of whole human brain and human brain tumor cell line total protein. CNS, central nervous system; LC-MS/MS, liquid chromatography-tandem mass spectrometry.

| Test | Protein name | Accession No. |
|------|--------------|---------------|
| 1    | 3-hydroxyacyl-CoA dehydrogenase type-2 | NP_004484.1 |
| 1    | 40S ribosomal protein S24 isoform c | NP_001017.1 |
| 1    | 40S ribosomal protein S5 | NP_001000.2 |
| 1    | Actin, α skeletal muscle | NP_001091.1 |
| 1    | ATP synthase subunit β, mitochondrial precursor | NP_001677.2 |
| 1    | ATPase family AAA domain-containing protein 3 A isoform 1 | NP_060658.3 |
| 1    | ATP-dependent RNA helicase DDX1 | NP_004930.1 |
| 1    | DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3 | NP_001347.3 |
| 1    | DNA-(apurinic or apyrimidinic site) lyase | NP_001632.2 |
| 1    | Nuclear factor 1 A-type isoform 2 | NP_005586.1 |
| 1    | Polyadenylate-binding protein 4 isoform 2 | NP_003810.1 |
| 1    | Replication protein A 14 kDa subunit | NP_002938.1 |
| 1    | RNA-binding protein 1-4 | NP_006319.1 |
| 1    | Tubulin alpha-1A chain | NP_002940.2 |
| 2    | 14-3-3 protein /H9256/H9004 | NP_003397.1 |
| 2    | 39S ribosomal protein L12, mitochondrial precursor | NP_002940.2 |
| 2    | 40S ribosomal protein S12 | NP_001007.2 |
| 2    | Actin, cytoplasmic 1 | NP_001092.1 |
| 2    | α-enolase | NP_001419.1 |
| 2    | ATP-dependent RNA helicase A | NP_001348.2 |
| 2    | Creatine kinase B-type | NP_001814.2 |
| 2    | Elongation factor 1-α1 | NP_001393.1 |
| 2    | Glial fibrillary acidic protein isoform 1 | NP_002046.1 |
| 2    | Glucose-6-phosphate isomerase isoform 2 | NP_000166.2 |
| 2    | Glyceraldehyde-3-phosphate dehydrogenase | NP_002037.2 |
| 2    | Heat shock cognate 71 kDa protein isoform 1 | NP_006586.1 |
| 2    | Heat shock protein HSP 90-β | NP_031381.2 |
| 2    | Histone H2A type 1 | NP_003501.1 |
| 2    | Histone H4 | NP_003529.1 |
| 2    | Hypothetical protein LOC348262 | NP_097251.2 |
| 2    | Keratin, type I cytoskeletal 10 | NP_000412.3 |
| 2    | Keratin, type II cytoskeletal 1 | NP_006112.3 |
| 2    | L-lactate dehydrogenase A chain isoform 1 | NP_005557.1 |
| 2    | NEFA-interacting nuclear protein NIP30 | NP_079222.1 |
| 2    | Nuclear factor 1 C-type isoform 1 | NP_005588.2 |
| 2    | Peptidyl-prolyl cis-trans isomerase A | NP_066953.1 |
| 2    | Phosphoglycerate mutase 1 | NP_002620.1 |
| 2    | Probable ATP-dependent RNA helicase DDX5 | NP_004387.1 |
| 2    | Probable ATP-dependent RNA helicase DDX6 | NP_004388.2 |
| 2    | Programmed cell death protein 6 | NP_037364.1 |
| 2    | Pyruvate kinase isozymes M1/M2 isoform M2 | NP_002645.3 |
| 2    | Small nuclear ribonucleoprotein-associated proteins B and B' isoform B | NP_003082.1 |
| 2    | SWI/SNF complex subunit SMARCC1 | NP_003065.3 |
| 2    | T-complex protein 1 subunit eta isoform a | NP_006420.1 |
| 2    | THO complex subunit 4 | NP_005773.3 |
| 2    | U1 small nuclear ribonucleoprotein 70 kDa | NP_003080.2 |
| 1, 2 | 60 kDa heat shock protein, mitochondrial | NP_002147.2 |
| 1, 2 | Proteasome activator complex subunit 3 isoform 1 | NP_005780.2 |
| 1, 2 | Ribonuclease P protein subunit p30 isoform b | NP_006404.1 |
| 1, 2 | Tubulin β chain | NP_821133.1 |

*a* These numbers show in which 2 LC-MS/MS shotgun analyses the proteins were identified.

*b* Protein identification was based on the criterion of having at least one MS/MS data with Mascot scores that exceeded the thresholds (p < 0.05).
DISCUSSION

The major findings of this study were the following: (i) associations between SLE and several new and previously reported autoantigens/autoantibodies were demonstrated using multiple proteomic analyses: two-dimensional-PAGE/WB/MALDI-TOF/MS analysis and immunoprecipitation followed by LC-MS/MS shotgun analysis; and (ii) statistically significant associations between anti-esterase D antibodies and CNS syndromes as well as between anti-APEX nuclease 1 antibodies and psychiatric disorders in SLE were also shown by ELISAs, although the associations of other autoantibodies with CNS syndromes were unexpectedly unclear.

We found new, previously unreported associations of three serum autoantibodies with SLE, demonstrating reactivity against crystallin αB, esterase D, and APEX nuclease 1. Crystallin αB is a small stress protein with cytoprotective and anti-apoptotic functions that is abundant in the eye lens, but its constitutive expression at high levels is otherwise restricted to a limited number of tissues with high oxidative function, such as myocardial and skeletal muscle (31). Humoral responses against crystallin αB have been observed in patients with inflammatory nervous system diseases and Guillain-Barré syndrome, implying it has a role in the pathophysiology of these disorders. However, we could find no previous report of a specific association between crystallin αB and SLE in the literature. Human esterase D (carboxylesterase) is one member of the nonspecific esterase family defined by their reaction with synthetic ω-acyl ester substrates (32). It has been found in most tissues, but the highest levels of this enzyme are found in the liver and the kidney. It has been suggested that esterase D may have a role in detoxification. Autoantibodies against esterase D produced in experimental autoimmune uveoretinitis mice were also detected in some endogenous uveitis human patients (33). However, we also found no previous reports of a specific association between esterase D and SLE or CNS syndromes. APEX nuclease 1 (also known as multifunctional DNA repair enzyme 1) is a mammalian major apurinic/apyrimidinic endonuclease that plays a central role in DNA base excision repair (34). It is also a redox factor, stimulating the DNA-binding activity of several transcription factors. APEX is expressed throughout development in all tissues and its expression is sensitive to changes in cellular conditions including oxidative stress, wound healing, and hypoxia. In the present study, anti-APEX nuclease 1 antibodies were specifically detected in the sera of SLE patients, but not in other diseases or in NHCs. Again, we found no previous reports in the literature of an association between APEX nuclease 1 and SLE or CNS syndromes.

Our proteomic analyses also identified two autoantibodies with previously reported associations with SLE: ribosomal protein P0 and proteasome activator complex subunit 3 (PA28γ/K). Several other established SLE-associated autoan-
tigens, such as Sm and SS-A/Ro, were also identified by our proteomic screening. These results attest to the reliability of the immunoproteomic approach used in this study for identifying autoantigens in SLE. Phosphorylated ribosomal (P ribosomal) proteins are three ubiquitous, highly conserved acidic phosphoproteins (P0, P1, and P2) that play roles in protein synthesis (35). The ribosomal protein P0 localizes to the membrane surface of neuronal, hepatic, and endothelial cells in an immunologically accessible way. Antibodies to the P ribosomal proteins are considered highly specific markers for SLE and appear to correlate with disease activity for the liver and kidney disease as well as with CNS involvement. Proteasomes were originally described as cytoplasmic, ATP-dependent, proteolytic enzyme complexes involved in antigen presentation on MHC class I molecules. More recent structural analyses, however, show the nuclear antigen K, (PA28γ) to be an element of proteasomes. Moreover, studies examining the biological function of the proteasome show that it also catalyzes proteolysis for metabolism and protein quality control, apoptosis, signal transduction, and cell-cycle regulation. Recent advances in immunohistology have revealed that some neurodegenerative diseases, including Alzheimer’s dis-

Fig. 3. Representative results of ELISAs using sera from SLE patients against recombinant autoantigens. Serum IgG autoantibodies against the 11 selected recombinant autoantigens were validated by ELISA using serum samples from 106 SLE patients, including 42 patients with active CNS syndromes, and 100 NHCs. Positive results are shown for (A) Crystallin αB, (B) Esterase D, (C) APEX nuclease 1, (D) 60S acidic ribosomal protein P0 (ribosomal protein P0), and (E) Proteasome activator complex subunit 3 (PA28γ). Titers for the autoantibodies are expressed as the mean O.D. values of the triplicate wells. p values were calculated using the Mann-Whitney U test.

Fig. 4. Autoantibodies against selected antigens in the sera of patients with SLE and other autoimmune diseases as assessed by ELISA. The disease specificities of selected autoantibodies were assessed by ELISA using sera from patients with RA, SSc, SS, or MS. Titers for the autoantibodies were expressed as the mean O.D. values of the triplicate wells. The ELISA O.D. values for the sera of SLE patients were significantly higher than those of patients with other diseases or the NHC for (A) APEX nuclease 1 (SLE versus all the other controls by the Steel multiple comparison test; p < 0.0001), but did not differ significantly for (B) Crystallin αB. The horizontal dotted line in Fig. 4A indicates a theoretical cutoff titer of 0.39 (O.D.) for anti-APEX nuclease 1 antibodies determined as the NHC mean +3SD, as described in the Results section.
ease and Parkinson’s disease, may result from a dysfunction of the ubiquitin-proteasome system. Antibodies to Ki (PA28/H9253) have been reported in some patients with SLE. Statistically significant associations between anti-esterase D antibodies and CNS syndromes as well as between anti-APEX nuclease 1 antibodies and psychiatric disorders in SLE were also demonstrated for the first time. However, although promising, these novel findings require cautious interpretation and further investigation to allow for decisive conclusions because most of the O.D. values of anti-esterase D antibodies in the present study were not very high and there were several samples from patients without psychiatric disorders that had high-titer anti-APEX1 antibodies and vice versa. In addition, the biological relationship between these ubiquitous proteins and CNS-specificity was not clear in the present study. In the assessment of other antibodies, although WB analyses with smaller sample sizes showed significant specificity, most of the ELISA results with larger sample sizes indicated that they had insufficient specificity to be useful biomarkers for CNS lupus. The reason for these conflicting results is unclear, but may be related to the heterogeneity of patients with CNS lupus or methodological differences between WB and ELISA. It is worth mentioning that various forms of CNS lupus syndromes were pooled, which could confound the results of the present study. In addition, the ubiquitous proteins examined in the present study are probably less specific for CNS syndrome-related autoantigens. More promising results may be obtained by focusing on specific antigens localized exclusively in the brain.

In conclusion, the association of SLE with several new and previously reported autoantibodies was demonstrated using 2 proteomic approaches. Among them, statistically significant associations between anti-esterase D antibodies and CNS syndromes as well as between anti-APEX nuclease 1 antibodies and psychiatric disorders in SLE were also shown. This study demonstrates that the immunoproteomic approach used in this study combining two-dimensional-PAGE/WB/MALDI-TOF/MS analysis and immunoprecipitation followed by LC-MS/MS shotgun analysis used in the present study has rarely been applied for systemic autoimmune diseases such as SLE so far. Moreover, there are disadvantages of two-dimensional-PAGE/WB/MALDI-TOF/MS analysis including poor reproducibility, lack of sensitivity, low throughput, and a considerable workload. Several approaches to address the limitations of two-dimensional-PAGE/WB/MALDI-TOF/MS analysis have recently been introduced. However, immunoprecipitation followed by LC-MS/MS shotgun analysis used in the present study has rarely been applied for systemic autoimmune diseases such as SLE so far. Although many protein spots were found by two-dimensional-PAGE/WB/MALDI-TOF/MS analysis in our study, most were difficult to identify for several reasons. First, too many minor proteins in each spot could not be identified because of the limited sensitivity of the method. Second, the sera of active SLE patients usually contain many high-titer, high-affinity polyclonal antibodies. Finally, these antibodies often recognized minor proteins compared with cancer-related proteins. In contrast, more than 150 proteins were identified using immunoprecipitation followed by LC-MS/MS shotgun analysis. Thus, when samples that include many high-titer polyclonal antibodies, such as those found in the sera of active SLE patients, are tested for autoantigens, the immunoproteomic approach used in this study combining two-dimensional-PAGE/WB/MALDI-TOF/MS analysis and immunoprecipitation followed by LC-MS/MS shotgun analysis is an effective and reliable method.

In conclusion, the association of SLE with several new and previously reported autoantibodies was demonstrated using 2 proteomic approaches. Among them, statistically significant associations between anti-esterase D antibodies and CNS syndromes as well as between anti-APEX nuclease 1 antibodies and psychiatric disorders in SLE were also shown. This study demonstrates that the immunoproteomic approach used in this study combining two-dimensional-PAGE/WB/MALDI-TOF/MS analysis and immunoprecipitation followed by LC-MS/MS shotgun analysis is a reliable and effective method for the identification of autoantigens in SLE. Determining the precise roles of newly identified SLE-related au-
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toantibodies in the pathogenesis of SLE and their usefulness as biomarkers will require further study.

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REFERENCES
1. Kurien, B. T., and Scofield, R. H. (2006) Autoantibody determination in the diagnosis of systemic lupus erythematosus. Scand. J. Immunol. 64, 227–235
2. Giles, I., and Putterman, C. (2008) Autoantibodies and other biomarkers - pathological consequences (1). Lupus 17, 241–246
3. Rahman, A., and Isenberg, D. A. (2008) Systemic lupus erythematosus. N. Engl. J. Med. 358, 929–933
4. Hanly, J. G., and Harrison, M. J. (2005) Management of neuropsychiatric lupus. Best Pract. Res. Clin. Rheumatol. 19, 799–821
5. ACR Ad Hoc Committee on Neuropsychiatric Lupus Nomenclature (1999) The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. Arthritis Rheum. 42, 599–608
6. Nishimura, K., Harigai, M., Omori, M., Sato, E., and Hara, M. (2008) Blood-brain barrier damage as a risk factor for corticosteroid-induced psychiatric disorders in systemic lupus erythematosus. Psychoneuroendocrinology 33, 395–403
7. Colasanti, T., Delunardo, F., Margutti, P., Vacirca, D., Piro, E., Siracusano, A., and Ortona, E. (2009) Autoantibodies involved in neuropsychiatric manifestations associated with systemic lupus erythematosus. J. Neurol. 212, 3–9
8. Piebani, M., Pلطtni, M., Celadini, M., Bernardi, D., and Mion, M. M. (2009) Recent advances in diagnostic technologies for autoimmune diseases. Autoimmun. Rev. 8, 238–243
9. Hueber, W., and Robinson, W. H. (2006) Proteomic biomarkers for autoimmune disease. Proteomics 6, 4100–4105
10. Lefranc, D., Launay, D., Dubucquoi, S., de Seje, J., Dussart, P., Vermersch, M., Hachulla, E., Haton, P. Y., Vermersch, P., Moutonh, L., and Prin, L. (2007) Characterization of discriminant human brain antigen targets in neuropsychiatric systemic lupus erythematosus using an immunoproteomic approach. Arthritis Rheum. 56, 3420–3432
11. Suzuki, M., Ross, G. F., Wiers, K., Nelson, S., Bennett, M., Passo, M. H., Devarajans, P., and Brunner, H. I. (2007) Identification of a urinary proteomic signature for lupus nephritis in children. Pediatr. Nephrol. 22, 2047–2057
12. Kimura, A., Sakurai, T., Tanaka, Y., Hozumi, K., Takahashi, K., Takemura, M., Saito, K., Seishima, M., and Inuzuka, T. (2008) Proteomic approach. J. Proteome Res. 7, 116–121
13. Takagi, K., Tanaka, M., Ichida, H., Tochimoto, A., Kanno, T., Nishimura, K., Kamatani, N., and Hara, M. (2007) Diagnostic reliability of cerebral spinal fluid tests for acute confusional state (delirium) in patients with systemic lupus erythematosus: interelectro 6 (IL-6), IL-8, interferon-alpha, IgG index, and Q-albumin. J. Rheumatol. 34, 2010–2017
14. Hayashi, Y., Harigai, M., Kawaguchi, Y., Fukasawa, C., Soejima, M., Takagi, K., Tanaka, M., Ichida, H., Tochimoto, A., Kanno, T., Nishimura, K., Kamatani, N., and Hara, M. (2010) Diagnostic reliability of magnetic resonance imaging for central nervous system syndromes in systemic lupus erythematosus: a prospective cohort study. BMC Musculoskelet. Disord. 11, 13
15. Arnett, F. C., Edworthy, S. M., Bloch, D. A., McShane, D. J., Fries, J. F., Cooper, N. S., Healey, L. A., Kaplan, S. R., Liang, M. H., Luthra, H. S., and et al. (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 31, 315–324
16. Vitali, C., Bombardieri, S., Moutsopoulos, H. M., Coll, J., Geri, R., Hartron, P. Y., Kater, L., Konttininen, Y. T., Mamtorpe, R., Meyer, O., Mosca, M., Ostuni, P., Pellerito, R. A., Pennec, Y., Porter, S. R., Richards, A., Sauveze, B., Schiott, M., Sciuto, M., Shoenfeld, Y., Skopoulis, F. N., Smolen, J. S., Sorenmeno, F., Tisher, M., Wattiaux, M. J. (1996) Assessment of the European classification criteria for Sjögren’s syndrome in a series of clinically defined cases: results of a prospective multicentre study. The European Study Group on Diagnostic Criteria for Sjögren’s Syndrome. Ann Rheum Dis 55, 116–121
17. Polman, C. H., Reingold, S. C., Edan, G., Filippi, M., Kurtz, H. P., Kappos, L., Lublin, F. D., Metz, L. M., McFarland, H. F., O’Connor, P. W., Sandberg-Wolleheim, M., Thompson, A. J., Weizenhiner, B. G., and Wolinsky, J. S. (2005) Diagnostic criteria for multiple sclerosis: 2005 revisions to the “McDonald Criteria”. Ann. Neurol. 58, 840–846
18.龙头ia, D., and Kimura, N. (1997) Standardization of protocol for Immobilization 2-D PAGE and construction of 2-D PAGE protein database on World Wide Web home page. Jpn. J. Electrothor. 41, 13–20
19. Gires, O., Munz, M., Schaffhnri, M., Kieu, C., Rauch, J., Ahlmann, M., Eberle, D., Mack, B., Wollenberg, B., Lang, S., Hofmann, T., Hammer-schmidt, W., and Zeidler, R. (2004) Profile identification of disease-associated humoral antigens using AMIDA, a novel proteomics-based technology, Cell Mol Life Sci 61, 1198–1207
20. Kratimirceva, I., Blagove, B., Haack-Sorensen, K., Kassem, M., and Mann, M. (2005) Mechanism of divergent growth factor effects in mesenchymal stem cell differentiation. Science 308, 1472–1477
21. Philip, R., Murthy, S., Kavakov, J., Sinnathamby, G., Zerfas, D., and Philip, M. (2007) Shared immunoprofiling for ovarian cancer diagnostics and immunotherapy: potential theranostic approach to cancer. J. Proteome Res. 6, 2509–2517
22. Oyama, M., Kozuka-Hata, H., Tasaki, S., Setiba, K., Hattori, S., Sugano, S., Inoue, J., and Yamamoto, T. (2009) Temporal perturbation of tyrosine phosphoproteins dynamics reveals the system-wide regulatory network. Mol. Cell Proteomics 8, 226–231
23. Oyama, M., Itagaki, C., Hata, H., Suzuki, Y., Izumi, T., Natsume, T., Isobe, N., Oury, T., Vandersteen, D., Cenlens, P., Camacho, C. J., Weinberg, A., and Ascherman, D. P. (2007) Species-specific immune responses generated by histidyl-RNA synthetase immunization are associated with
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31. Wanschitz, J., Ehling, R., Löscher, W. N., Künz, B., Deisenhammer, F., Kuhle, J., Budka, H., Reindl, M., and Berger, T. (2008) Intrathecal anti-alphaB-crystallin IgG antibody responses: potential inflammatory markers in Guillain-Barre syndrome. J Neurol 255, 917–924

32. Lee, W. H., Wheatley, W., Benedict, W. F., Huang, C. M., and Lee, E. Y. (1986) Purification, biochemical characterization, and biological function of human esterase D. Proc. Natl. Acad. Sci. U.S.A. 83, 6790–6794

33. Okunuki, Y., Usui, Y., Kezuka, T., Hattori, T., Masuko, K., Nakamura, H., Yudoh, K., Goto, H., Usui, M., Nishioka, K., Kato, T., and Takeuchi, M. (2008) Proteomic surveillance of retinal autoantigens in endogenous uveitis: implication of esterase D and brain-type creatine kinase as novel autoantigens. Mol. Vis. 14, 1094–1104

34. Ikeda, S., Ayabe, H., Mori, K., Seki, Y., and Seki, S. (2002) Identification of the functional elements in the bidirectional promoter of the mouse O-sialoglycoprotein endopeptidase and APEX nuclease genes. Biochem. Biophys. Res. Commun. 296, 785–791

35. Brianì, C., Lucchetta, M., Ghirardello, A., Toffanin, E., Zampieri, S., Ruggero, S., Scarlato, M., Quattarini, A., Bassi, N., Ermani, M., Battistin, L., and Doria, A. (2009) Neurolupus is associated with anti-ribosomal P protein antibodies: an inception cohort study. J. Autoimmun. 32, 79–84

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