

# The Pathogenesis of the Demyelinating Form of Guillain-Barre Syndrome (GBS): Proteo-peptidomic and Immunological Profiling of Physiological Fluids\*<sup>§</sup>

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Acute inflammatory demyelinating polyneuropathy (AIDP) – the main form of Guillain-Barre syndrome—is a rare and severe disorder of the peripheral nervous system with an unknown etiology. One of the hallmarks of the AIDP pathogenesis is a significantly elevated cerebrospinal fluid (CSF) protein level. In this paper CSF peptidome and proteome in AIDP were analyzed and compared with multiple sclerosis and control patients. A total protein concentration increase was shown to be because of even changes in all proteins rather than some specific response, supporting the hypothesis of protein leakage from blood through the blood-nerve barrier. The elevated CSF protein level in AIDP was complemented by activation of protein degradation and much higher peptidome diversity. Because of the studies of the acute motor axonal form, Guillain-Barre syndrome as a whole is thought to be associated with autoimmune response against neurospecific molecules. Thus, in AIDP, autoantibodies against cell adhesion proteins localized at Ranvier's nodes were suggested as possible targets in AIDP. Indeed, AIDP CSF peptidome analysis revealed cell adhesion proteins degradation, however no reliable dependence on the corresponding autoantibodies levels was found. Proteome analysis revealed overrepresentation of Gene Ontology groups related to responses to bacteria and

virus infections, which were earlier suggested as possible AIDP triggers. Immunoglobulin blood serum analysis against most common neuronal viruses did not reveal any specific pathogen; however, AIDP patients were more immunopositive in average and often had polyinfections. Cytokine analysis of both AIDP CSF and blood did not show a systemic adaptive immune response or general inflammation, whereas innate immunity cytokines were up-regulated. To supplement the widely-accepted though still unproven autoimmunity-based AIDP mechanism we propose a hypothesis of the primary peripheral nervous system damaging initiated as an innate immunity-associated local inflammation following neurotropic viruses egress, whereas the autoantibody production might be an optional complementary secondary process. *Molecular & Cellular Proteomics* 15: 10.1074/mcp.M115.056036, 2366–2378, 2016.

Guillain-Barre syndrome (GBS)<sup>1</sup> is a rare severe disorder of the peripheral nervous system (1, 2). The etiology of this

<sup>1</sup> The abbreviations used are: GBS, Guillain-Barre syndrome; AIDP, acute inflammatory demyelinating polyneuropathy; AMAN, acute motor axonal neuropathy; AMSAN, acute motor-sensory axonal neuropathy; BBB, blood brain barrier; BNB, blood nerve barrier; CAM, cell adhesion molecule; CMV, cytomegalovirus; CNS, central nerve system; CNTN2, contactin 2; CSF, cerebrospinal fluid; DRG, dorsal root ganglia; EAE, experimental allergic encephalomyelitis; EAN, experimental allergic neuritis; EBV, Epstein-Barr virus, EBV-EA, Epstein-Barr virus early antigen; EBV-NA, Epstein-Barr virus nuclear antigen; EBV-VCA, Epstein-Barr virus viral capsid antigen; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; GO, gene ontology; HHV, human herpesvirus; HRP, horseradish peroxidase; HSV, herpes simplex virus; HPLC, high performance liquid chromatography; IFN- $\gamma$ , interferon- $\gamma$ ; IgGs, immunoglobulins G; KEGG, Kyoto Encyclopedia of Genes and Genomes; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LMP, 1 Epstein-Barr virus latent membrane protein 1; MBP, myelin basic protein; MCP-1, monocyte chemoattractant protein 1; MIP-1 $\beta$ , macrophage inflammatory protein-1 $\beta$ ; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; NCS, nerve conduction study; NFASC, neurofascin; NRCAM, neuronal cell adhesion molecule; PNS, peripheral nerve system; TGF $\beta$ , transforming growth factor beta; VZV, varicella-zoster virus.

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disease has remained an intriguing question for decades (2, 3). The first description of the symptoms of the disease, including ascending paralysis, was reported by Landry in 1859 (4) and further elaborated in 1916 when Guillain, Barre, and Strohl performed a more sophisticated description of this abnormality of the neural system (5). The development of experimental allergic neuritis (EAN) by injecting peripheral nervous tissue together with Freund's adjuvant into rabbits disclosed some similarities with the autoallergic symptoms of GBS, which finally allowed attributing this disease to neuroautoimmunity (6). The autoimmune features of GBS were further supported by Asbury *et al.* in their autopsy studies of GBS patients (7). More recently, precise cellular immunological and immunochemical studies of EAN models allowed researchers to partially uncover the mechanisms of GBS development and pathogenesis (8, 9). Comparable methodologies have described the induction and immunological features of EAN as the GBS model of peripheral neurodegeneration and experimental allergic encephalomyelitis (EAE) as the model for multiple sclerosis (MS), which lead to neurodegeneration in the central nervous system and thereby provide additional opportunities for speculating about the etiology of these diseases (9, 10).

The overall incidence of GBS in Western countries has been estimated to range from 0.89–1.89 cases per 100,000 persons per year (11). GBS is defined as a paralytic demyelinating disorder that is accompanied by massive lymphocytic infiltration and damage to the myelin sheath of the peripheral nerves. GBS is a monophasic self-limiting disease, and most patients fully recover. Recurrence is observed in 3–10% of patients. There are at least four subtypes of GBS, which are differentiated by nerve electrophysiological characteristics, and they have different pathogenesis and appearances: acute inflammatory demyelinating polyradiculoneuropathy (AIDP); acute motor axonal neuropathy (AMAN); acute motor and sensory axonal neuropathy (AMSAN); and Miller Fisher syndrome (3). Although the precise incidence of each GBS subtype has not been elucidated, it is believed that AIDP accounts for about 90% of all GBS cases in Western countries (12).

According to the data reported by a number of studies, various viral and bacterial infections precede a considerable portion of GBS patients. *Campylobacter jejuni* (13, 14), Epstein-Barr virus (EBV), cytomegalovirus (CMV) (13, 15), and *Mycoplasma pneumoniae* (13) are among the most common infectious agents that are thought to be able to trigger GBS. Besides, the first strong evidence that Zika virus can cause GBS development was published recently (16). There are some suggestions regarding the recruitment of molecular mimicry mechanisms and cytokine stimulation in the pathogenesis of GBS (17, 18). *Campylobacter jejuni* is regarded as the most probable trigger of the axonal forms of GBS (*i.e.* AMAN and AMSAN). The antibodies to *Campylobacter jejuni* have an affinity for GM1 and GD1A gangliosides, which are

located in the paranodal areas and the nodes of Ranvier in peripheral nerves (19, 20). Final disruption of the functioning of ionic channels blocks transmission along the nerve fiber leading to muscle weakness and sensory dysfunction. It has been suggested that the environmental hypothesis of GBS induction strongly correlates with that of MS triggering, where LMP1 (the EBV characteristic antigen) was recently documented as a specific “molecular signature” in the immunological repertoire of MS patients (21). Nonetheless, the pathogenesis of the most prevalent AIDP form of GBS remains poorly understood.

One of the diagnostic signs of GBS, which was observed almost a century ago, is an elevated CSF protein level without an elevated cell count (*i.e.* albuminocytologic dissociation) (5). Usually, the CSF protein concentration is normal for the first few days of the disease, but begins to rise by the end of the first week, peaking at 4–6 weeks (22). Importantly, the CSF protein concentration is 100- to 200-fold lower than in blood (23, 24). This fact must considerably simplify the identification of neuro-specific proteins and their fragments in the CSF. Previously, the CSF proteome of GBS patients was studied using exclusively the 2D-PAGE technique in search for proteins specifically up- or downregulated in GBS patients (25–28). In total, <20 such proteins were identified in all those studies, and most were highly abundant plasma proteins. Taking into consideration the known limitations of the 2D-PAGE method (*e.g.* its limited dynamic range of detection and inapplicability toward highly acidic, highly basic, large, small, or membrane proteins), those results can hardly be regarded as comprehensive. Because the catabolic degradation of proteins depends on the physiological state of the tissue, we believe that the proteomic and peptidomic composition of the CSF from AIDP patients should provide useful information on the pathogenetic mechanisms. Our data demonstrate that, in contrast to control patients, the CSF from AIDP patients is enriched with peptides related to the proteins involved in the arrangement of the axonal domains, whereas the overrepresented in AIDP proteins are mainly linked with the defensive responses to bacteria. These observations are in line with the up-regulation of CSF cytokines associated with innate immunity. On the basis of our results as well as critically reconsidered previously published data we suggest a hypothesis for the AIDP pathogenesis without the leading role of autoimmune processes.

## MATERIALS AND METHODS

### MATERIALS

The following chemicals were used: sequencing-grade modified trypsin (Promega, Madison, WI), iodoacetamide, dithiothreitol (DTT), formic acid (FA) and trifluoroacetic acid (TFA) (Sigma-Aldrich, St. Louis, MO), glycine, tris-(hydroxymethyl)-aminomethane (Tris-HCl) (Panreac, Spain), and ammonium acetate (Fluka, Germany). Hypergrade-quality acetoni-

trile (ACN) for LC-MS LiChrosolv®, acetone for liquid chromatography (Lichrosolv®), gradient-grade methanol for liquid chromatography (LiChrosolv®), gradient-grade ethanol for liquid chromatography (LiChrosolv®), and HPLC-grade water were purchased from Merck (Darmstadt, Germany).

### PATIENTS

Sample names, descriptions, characteristics and grouping are listed in the [supplemental Table S1](#). The GBS group included 88 patients who received examinations and treatments at the Research Center of Neurology (Moscow, Russia). GBS was diagnosed according to criteria of the Brighton Collaboration GBS Working Group (29). The form of GBS was determined according to the neurophysiological classifications of GBS (30). All patients were examined by nerve conduction study (NCS) during the acute period of the disease as the neurological symptoms were rising, which was no later than 2 weeks after the beginning of the disease but strictly before therapy, repeated NCS was done 1 month later to confirm the diagnosis (31). Most patients were in poor or very poor condition (quadriplegia and/or artificial ventilation). In total, 68 of 88 GBS patients had AIDP, and the remaining 20 GBS patients had the AMAN/AMSAN form.

The MS group included 13 patients who received examinations and treatments at the Research Center of Neurology. All patients were examined and diagnosed by a neurologist, fulfilled the McDonald criteria (32), and were classified with relapsing-remitting multiple sclerosis (RRMS). A sample from a patient with a single demyelinating event—so-called clinically isolated syndrome (CIS)—was also included in the study.

The meningitis group included 7 patients with microbiologically proven pyogenic (bacterial) meningitis and 11 patients with aseptic (viral) meningitis (proven by the viral culture and the PCR analysis). Analyses were performed at A.I. Evdokimov Moscow State University of Medicine and Dentistry.

The control group, which was enrolled in all experiments and provided CSF samples, included 20 nonneurological, non-oncological patients from the Treatment and Rehabilitation Center of Ministry of Health of the Russian Federation. All control patients underwent surgical procedures (knee endoprosthesis replacement and transurethral resection of the prostate with the use of spinal anesthesia). The control group, which was enrolled in all ELISA experiments, included 20 healthy volunteers.

The study was approved by the ethics committees of the corresponding hospitals, and all patients provided written informed consent for their participation.

**CSF Samples**—CSF samples were obtained from the GBS, MS and control patients under sterile conditions using standard lumbar puncture techniques. The intervertebral foramen between the third and fourth lumbar vertebrae was chosen. A 0.5% solution of Novocain was used as the local anesthesia. After needle administration and mandrin removal, the cerebrospinal liquid (1 ml) obtained from the middle portion was

collected in a dry clean tube without preservatives. The material was taken from the control patients before the administration of the spinal anesthetic.

**Serum Samples**—Blood samples were collected in a red-top glass tubes that contained no preservatives or anticoagulants, allowed to clot at room temperature for 1 h, and centrifuged at room temperature at  $1200 \times g$  for 10 min. The obtained sera were divided into aliquots, immediately frozen, and stored at  $-80^\circ\text{C}$  until use.

**Peptide Isolation**—Peptides from the CSF were isolated using solid-phase extraction using Discovery DSC-18 reversed-phase cartridges (1 ml tubes; 50 mg) (Supelco, Bellefonte, PA). The CSF (500  $\mu\text{l}$ ) was heated in a boiling water bath for 10 min, cooled to room temperature, diluted twice with 0.1% aqua TFA, and applied to a preconditioned SPE cartridge. The cartridge was washed with 0.1% aqua TFA (1 ml) and eluted with 80% ACN in 0.1% aqua TFA (1 ml). The eluate was vacuum-dried and the obtained peptide material was dissolved in a solvent containing 5% ACN, 95%  $\text{H}_2\text{O}$  and 0.1% TFA (20  $\mu\text{l}$ ). Before LC-MS/MS analysis the samples were treated in an ultrasound bath for 2 min.

**Protein Assay**—The protein concentrations in the CSF samples were determined using the Quick Start Bradford protein assay (Bio-Rad Laboratories, Hercules, CA 94547, USA). The protein concentration was measured according to the standard protocol. Bovine serum albumin was used as the calibration standard.

**SDS-PAGE**—For the proteome analysis, the CSF samples were prefractionated by SDS-PAGE on a 14% running gels. The pooled CSF samples (200  $\mu\text{g}$  of each sample) were run on a large (20  $\times$  20 cm) gel, and the obtained bands were divided into 14 slices ([supplemental Fig. S1](#)). The individual CSF samples from AIDP patients (50  $\mu\text{g}$  of each sample) were run on a small (8  $\times$  8 cm) gel, and the obtained bands were divided into 4 slices ([supplemental Fig. S2](#)). Each slice was subjected to in-gel tryptic digestion followed by LC-MS/MS analysis.

**In-gel Tryptic Digestion of the Proteins**—Gel slices were excised into small pieces (1  $\times$  1 mm) and transferred into sample tubes. The protein disulfide bonds were reduced using 10 mM DTT (in 100 mM ammonium acetate) at  $56^\circ\text{C}$  for 30 min and alkylated with 55 mM iodoacetamide (in 100 mM ammonium acetate) at room temperature for 20 min in the dark. Then the gel samples were destained with 50% ACN (in 50 mM ammonium acetate) and dehydrated by the addition of 100% ACN. After the removal of ACN, the samples were subjected to the in-gel digestion. The digestion buffer solution contained 13 ng/ $\mu\text{l}$  trypsin in 50 mM ammonium bicarbonate. The trypsin digestion proceeded overnight at  $37^\circ\text{C}$ . The resulting tryptic peptides were extracted from the gel by adding two volumes (in comparison to digestion buffer solution) of 0.5% aqua TFA into the samples and incubating for 1 h. Then an equal volume of ACN was added and the samples were incubated for another 1 h. The extracted peptides were vac-

uum-dried and dissolved in a solution containing 5% ACN and 0.1% aqua TFA before the LC-MS/MS analyses.

**LC-MS/MS and Data Analyses**—LC-MS/MS analysis was performed using TripleTOF 5600+ mass spectrometer with a NanoSpray III ion source (AB Sciex, Concord, Ontario, Canada) coupled with a NanoLC Ultra 2D+ nano-HPLC system (Eksigent, Dublin, CA). The HPLC system was configured in a trap-elute mode. A mixture of 98.9% water, 1% methanol, and 0.1% formic acid (v/v) was used as the sample loading buffer and buffer A. Buffer B was 99.9% ACN, 0.1% formic acid (v/v). Samples were loaded on a Chrom XP C18 trap column (3  $\mu$ m, 120 Å, 350  $\mu$ m $\times$ 0.5 mm; Eksigent) at a flow rate of 3  $\mu$ l/min for 10 min and eluted through a 3C18-CL-120 separation column (3  $\mu$ m, 120 Å, 75  $\mu$ m $\times$ 150 mm; Eksigent) at a flow rate of 300 nl/min. The gradient was from 5 to 40% of buffer B within 120 min. The column and trap column were regenerated between runs by washing with 95% buffer B for 7 min and equilibrated with 5% buffer B for 25 min. Between different samples, a blank 45-min run consisting of 5 gradient waves (from 5% to 95% buffer B in 2 min; 95% buffer B for 5 min; and from 95% to 5% buffer B in 2 min) was used to thoroughly wash the system and to prevent any possible crosstalk. This was followed by the system equilibration for 22 min with 5% buffer B.

Information-dependent mass spectrometry experiments included 1 survey MS1 scan followed by 50 dependent MS2 scans. The MS1 acquisition parameters included the following: the mass range for analysis and subsequent ion selection for MS2 analysis was 300–1250  $m/z$ , and the signal accumulation time was 250 ms. The ions for the MS2 analysis were selected based on the intensity with the threshold of 400 cps and charge state of 2–5. MS2 acquisition parameters included the following: the resolution of the quadrupole was set to preset UNIT (0.7 Da); the measurement mass range was 200–1800  $m/z$ ; and the optimization of the ion beam focus was set to obtain maximal sensitivity. The signal accumulation time was 50 ms for each parent ion. Collision-activated dissociation was performed using nitrogen gas with collision energy ramping from 25–55 V within the 50-ms signal accumulation time. The analyzed parent ions were sent to dynamic exclusion list for 15 s.

The peak lists from the MS/MS data were generated and recalibrated using ProteinPilot (version 4.5; ABSciex) and then searched against the UniProt human Protein knowledgebase (version 2013\_03, 150600 entries) using MASCOT (version 2.2.07) and X! Tandem (version CYCLONE-2013.2.01) in parallel. Precursor and fragment mass tolerances were set to 20 ppm and 0.04 Da, respectively. The database searches for peptidomic experiments included the following parameters: unspecific enzyme and dynamic/flexible methionine oxidation. For the proteomic experiments, we used the following database search parameters: enzyme - trypsin (maximum 1 missed cleavage), fixed modification for Cys (carbamidom-

ethylation), dynamic/flexible methionine oxidation, C-terminal amidation, and N-terminal Glu->pyro-Glu.

The peak lists obtained were also searched against the database for human immunoglobulin cDNA (version: November 2013, 98502 entries), which was downloaded from [http://www.imgt.org/ligmdb/\(33\)](http://www.imgt.org/ligmdb/(33)), using MASCOT (version 2.2.07) and X! Tandem (version CYCLONE-2013.2.01). The database was downloaded using the following query parameters: *Homo sapiens* (human) taxonomy species; cDNA molecular type; rearranged configuration type; constant, variable, diversity, joining, and conventional-with-leader gene type; the L-V-J-C- and L-V-D-J-C-sequences of the molecular entity type. Validation of the identification results and the meta-analysis were performed using Scaffold 4 (version 4.2.1) (Proteome Software, Inc., Portland, OR). To evaluate both peptides and protein hits, a 5% false discovery rate was used.

The lists of the identified peptides were compared with those published by Zougman *et al.* (34) and Holttä *et al.* (35), and only completely coincident amino acid sequences were taken into account.

CSF proteome data for multiple sclerosis was taken from Hyung *et al.* (36) The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (37) via the PRIDE partner repository (38) with the data set identifiers: PXD002911, 10.6019/PXD002911, PXD002884 and 10.6019/PXD002884.

**ELISA**—MaxiSorp 96-well plates (Nunc, Roskilde, Denmark) were coated with a test antigen solution (Imp1, MOG, MBP (production and purification was described previously (39)), Contactin, Neurofascin, or NrCAM (R&D Systems Inc., Minneapolis, MN 55413, USA)) in 100 mM carbonate buffer (pH 9.0; 5  $\mu$ g/ml in 50  $\mu$ l per well), incubated at 4 °C overnight, and washed with 3 portions of a wash buffer (PBS with 0.1% Tween-20 [pH 7.4], 300  $\mu$ l per well) (unless otherwise specified, this washing was performed after each step). The wells were then blocked with 250  $\mu$ l dry milk in carbonate buffer and incubated at 37 °C for 1 h. For the viral antigens (HSV-1,2, EBV-EA, EBV-NA, EBV-VCA, HHV-6, HHV-8, and CMV), we used antigen-coated and blocked plates (Vector-Best, Moscow, Russia). Serum samples were diluted in PBS containing 0.5% BSA (or dry milk) and 0.05% Tween 20. Respective positive control antibodies were used for each antigen (human Contactin-2/TAG1 antibody, anti-human NrCAM antibody, human Neurofascin antibody (R&D Systems Inc.), anti-MOG antibody (Santa Cruz Biotechnology, Inc., Dallas, Texas 75220, USA), anti-MBP antibody (Abcam, Cambridge, UK)). The plates were incubated with primary antibodies at 37 °C for 1 h. Then, 50  $\mu$ l of HRP-conjugated anti-human anti-Fc antibodies (1:5000) were added to each well (we used anti-human anti-lambda antibodies for NrCAM-binding detection), and the plates were incubated at 37 °C for 1 h again. After washing with five portions of the wash buffer, 50  $\mu$ l of tetramethyl benzidine (Amresco, Solon, OH) was added to each well, and the plates were placed in the dark for 5–15 min. The

reaction was stopped by adding 10% phosphoric acid (50  $\mu$ l per well). The OD450 values were measured on a Varioscan Flash microplate reader (Thermo Scientific, Waltham, MA). Antigen quality and purity were additionally tested by SDS-PAGE, ELISA and Western blot (Supplemental file #).

**Cytokine Analysis**—The CSF and the serum samples were simultaneously analyzed for 17 cytokines, including (with the respective limit of detection as reported by the manufacturer shown in pg/ml): IL-1 $\beta$  (0.6), IL-2 (1.6), IL-4 (0.7), IL-5 (0.6), IL-6 (2.6), IL-7 (1.1), IL-8 (1.0), IL-10 (0.3), IL-12 (p70) (3.5), IL-13 (0.7), IL-17 (3.3), interferon- $\gamma$  (IFN- $\gamma$ ) (6.4), tumor necrosis factor-alpha (TNF- $\alpha$ ) (6.0), granulocyte colony-stimulating factor (G-CSF) (1.7), granulocyte macrophage colony-stimulating factor (GM-CSF) (2.2), MCP-1 (1.1), and MIP-1 $\beta$  (2.4). This analysis was performed using the Bio-Plex Pro Human Cytokine 17-plex Assay (Bio-Rad Laboratories) according to the manufacturer's instructions. Briefly, 50  $\mu$ l of each 3-fold diluted sample and cytokine standard were mixed with antibody-conjugated magnetic beads (50  $\mu$ l) in a 96-well flat-bottom plate (Greiner, Frickenhausen, Germany) and incubated for 30 min. After washing the plate, 25  $\mu$ l of the biotinylated antibody solution (Bio-Rad Laboratories) was added to wells, followed by 30 min of incubation. Streptavidin-Phycoerythrin (Bio-Rad) was added to each well and incubated for 10 min. After the final wash, the beads were resuspended in 125  $\mu$ l of the assay buffer (Bio-Rad Laboratories) and analyzed on a Suspension Array System (Bio-Plex 200 System; Bio-Rad Laboratories). The cytokine concentrations were calculated by reference to a standard curve for each cytokine.

**Statistical Analysis**—Data were analyzed using Sigma-Plot 12.5 and Statistica 10 software. Differences in the cytokine levels, antigen binding, and viral seropositivity between different groups (MS, healthy patients, and GBS patients) were compared using the Student *t* test and nonparametric Kolmogorov-Smirnov and Mann-Whitney *U*-tests. If the measured values were under the detection range, they were replaced with the low limits of detection. Difference was considered reliable when confirmed by  $\geq 2$  tests. The relationship between variables was evaluated using the Spearman rank correlation test. A 2-sided *p* value < 0.05 was considered statistically significant. Graphics with simultaneous virus occurrence were plotted with the help of the Euler APE v3 software (40).

### RESULTS

**CSF Protein Concentration Analyses**—CSF protein level analysis results in our samples were in average in accordance with the literature data (12, 41) showing prominent increase in GBS (Fig. 1A and supplemental Fig. S3). However, the dispersion of protein concentration was very high with some samples showing the level lower than the average in the control. Comparison of the concentration results with the CSF sampling timing in the course of GBS symptoms development

(supplemental Table S1) showed that (in average) the closer the CSF sampling was to the peak of the disease symptoms the more frequently the protein concentration was increased, which was also in accordance with the earlier observations (42). However, the dispersion of the observed protein concentrations in the CSF of the AIDP patients was rather high, probably because of the different symptom dynamics and severity of the disease, but our sample library was not large enough to establish a reliable dependence. This effect must be always taken into account when the total protein content or individual proteins are analyzed in the CSF of GBS patients.

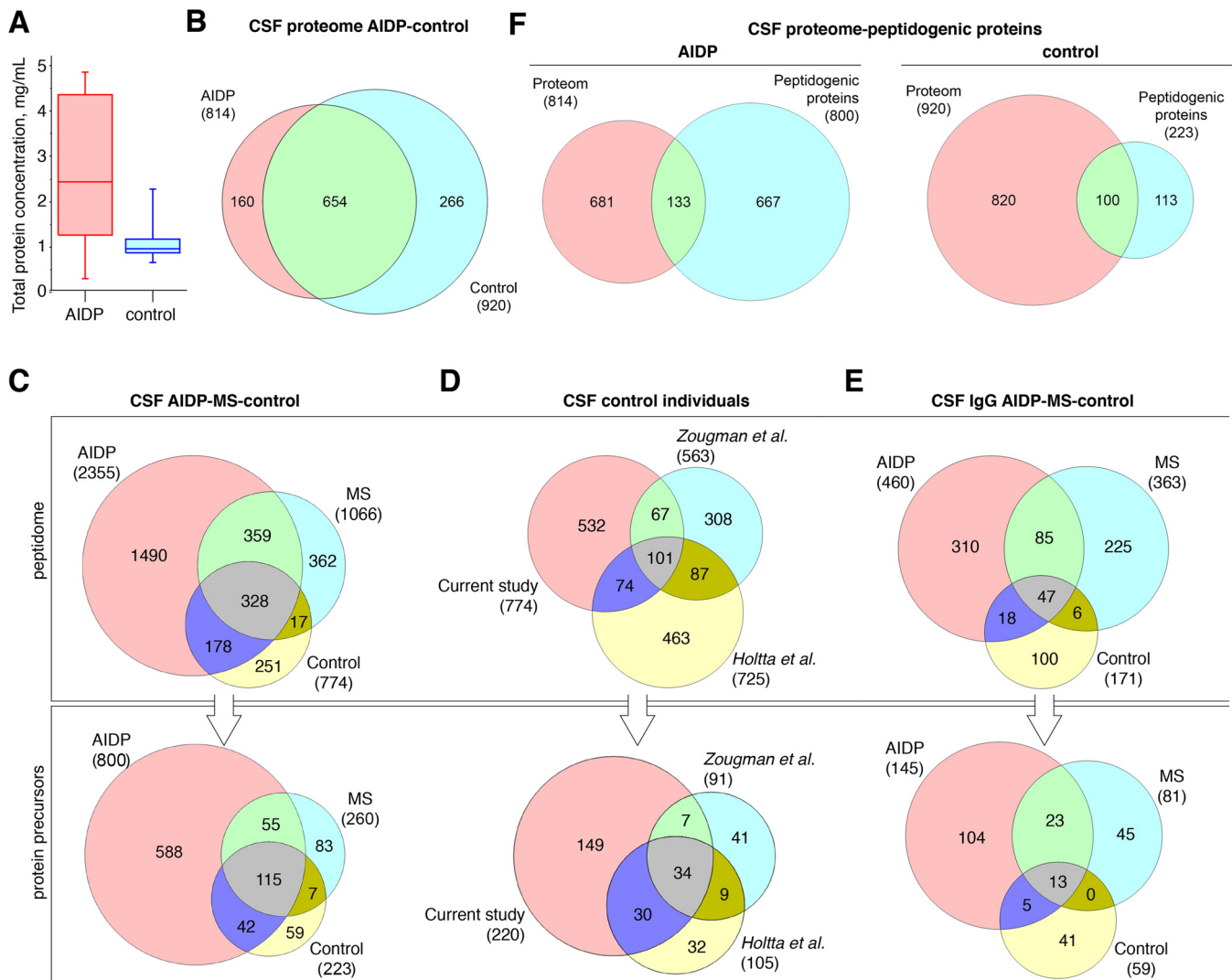
**Proteomic Profiling of the CSF Samples**—For proteome analysis the CSF samples were fractionated by SDS-PAGE with the subsequent in-gel trypsin digestion of each fraction and the LC-MS/MS analysis of extracted tryptic peptides. The individual CSF proteome analysis was made for two pairs of the AIDP patients, whose CSF samples were taken at different time intervals from the peak of neurological symptoms (see details in the supplemental Table S1). One pair of the samples (GBS14 and GBS16) had protein concentration <1 mg/ml and the other one (GBS02 and GBS03) had protein concentration around 4 mg/ml. In total 1064 proteins were identified in these samples, including 634 proteins in GBS02, 621 proteins in GBS03, 776 proteins in GBS14 and 747 proteins in GBS16 (supplemental Table S8, S9).

In the pooled CSF samples in total 854 proteins were identified, including 726 proteins in the control cohort and 542 proteins in the AIDP cohort (Fig. 1B, supplemental Table S2, S3).

Mapping the identified proteins in the KEGG-pathway database revealed a number of gene clusters differentially represented (*p* value < 0.05) in the AIDP, the MS (taken from (36)) and the control groups of the CSF samples (supplemental Fig. S4A, supplemental Table S6). A similar analysis using the Gene Ontology database also revealed a number of differentially represented biological processes (supplemental Fig. S4B, supplemental Table S7). For example, we found gene clusters associated with immune responses to infection, namely “complement and coagulation cascades,” “complement activation, lectin pathway,” and “lymphocyte chemotaxis” to be more represented in the AIDP proteome in comparison with the control group.

The correlation of the MS/MS spectra with the database of cDNA sequences for the human immunoglobulins (IgGs) [[http://www.imgt.org/ligmdb/\(33\)](http://www.imgt.org/ligmdb/(33))] revealed 1316 IgGs in the AIDP patients, and 921 IgGs in the Control group (supplemental Fig. S5, supplemental Tables S4 and S5).

**Peptidomic Profiling of the CSF Samples**—CSF peptidome was analyzed for the control patients (*n* = 20), MS patients (*n* = 6) and AIDP patients (*n* = 10), which resulted in the identification of 2958 unique peptides derived from 934 proteins. In particular, 2355 peptide fragments related to 800 proteins were found in the CSF samples from the AIDP patients, 1066 fragments of 260 proteins in the MS patients, and



**FIG. 1. A, Protein concentrations in the CSF of GBS and control patients. (B–F) Venn diagrams representing: B, proteins identified in the CSF of AIDP patients in comparison with control individuals; C, endogenous peptides and their protein precursors identified in the CSF of AIDP patients in comparison with MS and control patients; D, endogenous peptides and their protein precursors identified in the CSF of control patients in comparison with previously reported data; E, endogenous peptides corresponding to the IgG molecules identified in the CSF of AIDP patients in comparison with MS and control patients; and F, proteins and protein precursors of peptides identified in the CSF of AIDP patients and control individuals.**

774 fragments of 223 proteins in the Control group (supplemental Tables S10 and S11). Notably, the Venn diagrams demonstrate the lack of significant overlap between peptides and their protein precursors between the three groups of the samples (Fig. 1C). Detailed comparisons of the peptidomic profiles of the CSF samples obtained from the control patients with previously reported data (Fig. 1D) show only 29.8% overlap with the results of Zougman *et al.* (34) and 24.1% overlap with the data of Holtta *et al.* (35). The similarity of protein precursors was significantly higher –45% and 60.9%, respectively. It should be noted that many protein precursors were identified by a single peptide. We found ~100 such proteins, whereas Zougman *et al.* (34) and Holtta *et al.* (35) identified 34 and 29 such proteins, respectively. These values were 45.5%,

37.4%, and 27.6% of the total number of the identified proteins, respectively. The differences between the lists of the peptides identified in the current study and the previously reported ones, among others, may be explained by variations in the methods of peptide isolation and the mass spectrometry equipment.

According to the bioinformatics study, a considerable part of the peptidogenic proteins in supplemental Table S10 belong to the high abundant proteins of blood plasma. For the peptidogenic proteins, which cover the majority of the endogenous peptides, the number of peptides per protein is higher for the AIDP patients in comparison with the control group. Generally, this fact can be explained by the elevated concentration of proteins in the CSF of the AIDP patients, which was

mentioned earlier. Indeed, the increase in the content of the fibrinogen ( $\alpha/\beta$  chains), transthyretin, and albumin (these proteins are among the first 10 proteins according to the number of identified peptide fragments in [supplemental Table S11](#)) in the CSF during GBS development has been directly shown (43–45). However, we also observed comparatively large numbers of peptides derived from neuro-specific proteins in the CSF peptidome of the AIDP patients. As an example, the number of fragments of the VGF neurosecretory protein was as high as 119 in the AIDP patients (75% sequence coverage), whereas in the control group only 27 peptides (35% sequence coverage) were identified and 53 peptides (45% sequence coverage) were identified in the MS group. Moreover, the peptide fragments of the other neuro-specific proteins—in particular, the neuronal pentraxin receptor (7 peptide fragments in AIDP and 1 peptide in MS) and major prion protein (17 peptides in AIDP and 3 peptides in MS), which are required for peripheral myelin maintenance (46)—were exclusively found in the samples from the AIDP patients. Thus, the more likely explanation seems to be an enhanced catabolism of these proteins, rather than simple increase in the total protein concentration in the CSF.

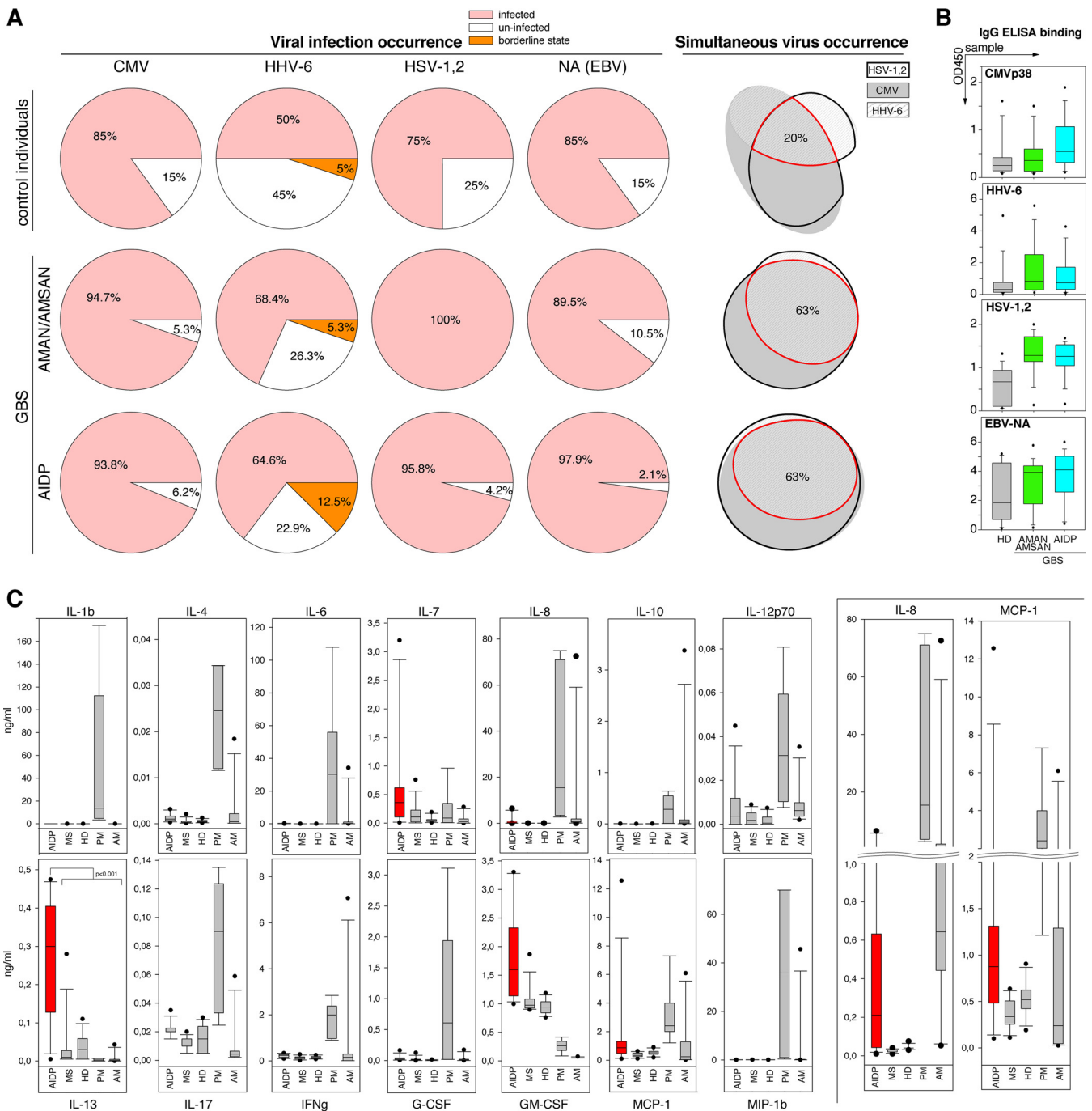
Another specific feature of the CSF peptidome during AIDP development in comparison with the control patients is an increase in the immunoglobulin fragments. In peptidomic experiments the correlation of the MS/MS spectra with the database of human immunoglobulins (IgGs) (33) revealed 460 peptide fragments from 145 various IgGs in the AIDP patients, 363 peptides from 81 IgGs in the MS patients, and 171 peptides from 59 IgGs in the control patients (Fig. 1E and [supplemental Table S12](#)). The antibody fragments observed may have resulted from the intracellular degradation of the antigen-antibody complexes (47), and an increase in the immunoglobulin fragments in the AIDP patients could reflect stimulation of their immune systems in comparison with the control individuals.

Mapping of the peptidogenic proteins in the KEGG ([supplemental Fig. S6A](#) and [supplemental Table S13](#)) and Gene Ontology ([supplemental Fig. S6B](#), [supplemental Tables S14–S16](#)) pathway databases revealed significant differences between the AIDP and the control individuals. Interestingly, the most reliably identified KEGG pathways ( $p$  value < 0.001), such as: “complement and coagulation cascades,” “staphylococcus aureus infection,” “ECM-receptor interaction,” and “Cell adhesion molecules (CAMs),” which might be attributed to immune system activation and deterioration of membrane proteins involved in cell-to-cell adhesion, were also significantly uprepresented in AIDP. Among those, the “cell adhesion molecule (CAM)” cluster attracts special attention. The peptidogenic proteins—in particular, neurofascin (NFASC), contactin 2 (CNTN2), and the neuronal cell adhesion molecule (NRCAM)—are unique to AIDP. One of the notable differences in the GO analysis was found for the “sequestering of TGF $\beta$  in the extracellular matrix” cluster. Fragments of two of three

proteins of this cluster (fibrillin-2 and the latent-transforming growth factor beta-binding protein 1 (LTBP)) were identified in the CSF of the AIDP patients, whereas none of them were found in the control individuals. Comparative peptidogenic and proteomic analyses of the CSF (Fig. 1F) clearly demonstrate that the relative occurrence of the CSF peptidogenic proteins in the corresponding proteomes significantly differed between the control and AIDP groups (20.1 and 45.3% of the total number of the identified peptidogenic proteins, respectively). In total, 639 peptidogenic CSF proteins were not identified in the proteomic experiments, and among these 115 were observed with no less than two unique peptide fragments. In conclusion, the CSF of the AIDP patients is enriched in products of protein degradation rather than in the proteins themselves.

*Immunological profiling of 88 GBS patients*—Although the crucial role of *Campylobacter jejuni* is well established for AMAN (48), for AIDP there is a questionable link with pathogenesis and different viral infections like CMV (49) or EBV (50). Importantly, peptidomic/proteomic profiling of CSF obtained from AIDP patients revealed evident signs of the proteins involved in antiviral defense. Therefore, we further analyzed the existence of the most common viral infections—CMV, EBV, HSV-1, 2, HHV-6 and HHV-8—in patients with different types of GBS (AIDP and AMAN/AMSAN) using seropositivity testing (Fig. 2). Our data suggest that the percentages of sera negative to CMV, HSV-1,2, or HHV-6 individuals were evidently higher in healthy donors in comparison with patients with either GBS type (Fig. 2A). There were no differences in the total EBV infection rates between healthy, AMAN/AMSAN, and AIDP patients. The IgG response against viral antigens was almost the same in all cohorts (Fig. 2B). Our results evidently demonstrate that none of the studied viruses are strictly necessary for AIDP progression, but at the same time we noted an elevated level of overall viral infection in GBS patients. Therefore, we compared the simultaneous presence of the three widespread and most common viruses—CMV, HHV-6, and HSV-1, 2—in healthy donors and GBS patients (Fig. 2A, right panel). Interestingly, 63% of AIDP and AMAN/AMSAN patients - in contrast to 20% of healthy individuals - were seropositive for all three viruses, thereby indicating that multiple simultaneous viral infections may contribute to the GBS triggering.

*Testing of serum autoantibodies specific to neuronal membrane proteins*—Previous reports elucidate the role of the GM1 and GD1a gangliosides as targets for autoimmune humoral responses in AMAN patients (51). Peptide fragments of neurofascin, NrCAM, and contactin were found in the CSF of AIDP patients. To test for possible autoantibodies, we performed ELISA analysis which, however, did not reveal statistically significant differences between control and pathological samples in serum IgG reactivity toward any of the tested neuronal membrane proteins, including MS autoantigens MBP and MOG ([supplemental Fig. S7](#)). Surface plasmon res-



**FIG. 2. Simultaneous multiviral infection persists in the majority of GBS patients.** *A*, Analysis of viral seropositivity in healthy patients (HD) and AMAN and AIDP patients studied using ELISA. Light pink, orange, and white indicate the percentages of infected individuals, individuals with borderline status, and uninfected persons, respectively. The plots on the right represent the percentage of individuals with multiple seropositive results for the indicated viruses. The gray area, crosshatched area, and area inside the bold line represent CMV, HHV-6, and HSV-1,2 seropositivity, respectively. The intersection area restricted by the red line corresponds to the simultaneous occurrence of respective viruses. The percentage of individuals who were seropositive toward all 3 viruses is indicated. *B*, Level of serum IgG specific for viral antigens measured by ELISA. *C*, Multiplex analysis of the cytokine level in the CSF of the AIDP patients, multiple sclerosis patients (MS), healthy individuals (HD), and patients with purulent (PM) and aseptic (AM) meningitis. The plots on the right demonstrate the scaled areas. Boxplots represent the interquartile range (25–75%), and the bars and dots indicate 90 and 95% confidential intervals, respectively. The boxplots filled with red indicate cytokines that were elevated in the CSF of the GBS patients in comparison with the healthy individuals.



onance studies confirmed that the serum antibodies from the AIDP patients did not bind MBP. Similarly, we failed to detect any catalytic activity of the antibodies against MOG and MBP (data not shown), the latter of which was previously shown to be hydrolyzed by autoantibodies in MS (52, 53).

**Cytokine Profiling of CSF**—To further characterize the AIDP-type GBS, we measured the cytokine levels in the CSF of AIDP patients and compared them with the levels in healthy donors, in patients with MS, which is a classic autoimmune neurodegenerative disease, and in patients with purulent and aseptic meningitis, which are typical inflammatory pathogen-driven CNS diseases (Fig. 2C and [supplemental Table S17](#)). We observed statistically significant elevations in IL-7, IL-8, GM-CSF, and IL-13 in AIDP in comparison with the healthy individuals and MS, of which IL-7, IL-13, and GM-CSF were elevated in AIDP in comparison with all the other tested pathologies. It is necessary to mention that in meningitis the level of up-regulated cytokines was increased up to 1000- to 10,000-fold in comparison with the healthy donors, whereas in AIDP the increase was only 2- to 25-fold. We further measured the serum cytokines levels in 5 AIDP patients to verify that the elevated cytokines did not simply penetrate through the blood brain barrier (BBB), but rather originated from the CNS or locally penetrated into CSF from inflamed regions. Multiplex analysis confirmed that none of the tested cytokines were up-regulated (data not shown). In conclusion, the cytokine profile of the AIDP CSF, in contrast to the meningitis, revealed a heterogeneous panel that generally was not directly associated with adaptive immune cells.

### DISCUSSION

In the present study we analyzed and critically revised different aspects of the pathogenesis of the AIDP, the prevailing form of the Guillain-Barre syndrome, which is characterized by the peripheral nerve demyelination and nerve conductivity damaging.

Because of the lack of knowledge on the CSF proteome in healthy population (e.g. normal proteome variability), restrictions in the CSF sampling, rarity of the disease, its very fast dynamics and impossibility to obtain samples from different patients at the same point of time in relation to the peak of neurological symptoms, our sample collection was far from adequate to make strict dynamic-specific observations. Besides, it was not possible to consider *a priori* at what stage of the disease the AIDP-specific changes in the CSF proteome might be most prominent. Moreover, not all samples were in enough quantities for both proteome and peptidome analysis. Thus, trying to resolve all these discrepancies in the CSF sampling and to detect both stage-unspecific and stage-specific changes in CSF proteome the main proteome analyses in this work was done on pooled CSF samples. Several individual CSF proteomes were also studied and the comparison of the lists of identified proteins showed that the closer to the peak of neurological symptoms of AIDP the CSF sample

was collected, the fewer proteins could have been identified ([supplemental Fig. S8](#)). However, the comparison of the results for the individual and pooled CSF samples showed that the number of AIDP specific proteins in all individual samples was almost the same. In other words, AIDP specific proteins might be already present in the CSF in the early stages of the disease, which justifies the way we pooled the samples. However, detailed stage-specific analysis is a task for future work.

For today the only generally accepted mechanism of GBS is that which employs autoantibodies against neuro-specific targets. Such mechanism was previously explicitly demonstrated in the axonal form of GBS - AMAN (at least in half of the AMAN cases), where an adaptive immune response against the bacteria *Campylobacter jejuni* led to antibody cross-reactivity against neuro-specific human gangliosides as a result of molecular mimicry. This same hypothesis was tried on all the other GBS forms including AIDP, however the results were not so clear. Here we suggest the modification of the generally accepted environmental (infection-triggered) hypothesis that does not require an autoimmune response for the peripheral nerve conductivity damaging to start.

One of the earliest known biochemical symptoms of GBS is an increase in the protein concentration in the CSF. And although other aspects of the GBS pathogenesis were extensively studied, this feature has not been thoroughly analyzed. Thus, the primary goal of this work was to analyze the CSF peptidomes and proteomes of AIDP patients, which were compared with those of the control patients without neurological diseases and with MS—the well-known autoimmune disease of CNS. Besides, different immunological aspects were also tested. Then the possible implications of the results regarding the autoimmune hypothesis of the GBS and, in particular, the AIDP were discussed.

The GBS is considered to affect only the peripheral nerve system, which normally is protected from the contact with antibodies and immune cells by the blood nerve barrier (BNB). In GBS, the protein content in the CSF increases, which is thought to be through the damage in the BBB and excessive blood protein infiltration. However, no details are known. At the same time there is a part of the PNS, particularly dorsal root ganglia and dorsal roots, where the blood microvessels are devoid of barrier properties (54) and which are in direct contact with the CSF in the subarachnoid cavity of the spinal cord. The pathomorphological picture of the GBS includes inflammatory infiltrates (a sign of the local activation of the immune system) around the peripheral nerve fibers, which mainly consist of lymphocytes and monocytes (7, 55). In addition, in an animal model of the spontaneous autoimmune peripheral polyneuropathy (presymptomatic L31/CD4<sup>-/-</sup> mice), the infiltration of immune cells was noted precisely in the areas of the DRG and spinal roots prior to disease onset (56). Thus, DRGs and dorsal roots theoretically might be the places of the excessive blood protein infiltration in the CSF. However, it also means that, in addition to blood proteins, the

products of the GBS-caused local inflammation (proteins, their degradation products, cytokines etc.) might also get into the CSF.

Despite the prominent increase in the CSF protein concentration in AIDP patients, cluster analysis of the proteomic data did not reveal any dramatic straightforward changes in a single protein group, but rather demonstrated dispersed minor changes that reflected the complexity of the responses and confirmed the theory of unspecific protein influx from the blood. At the same time, the most interesting findings were in the peptidome.

The first observation was that in the AIDP the total number of peptides in the CSF was greatly increased. Because there are few free peptides in blood (57), it was logically to suggest their local generation. Because there are no active inflammation processes in the CNS, these peptides must have been the result of the abovementioned local inflammation in the DRGs and dorsal roots. Also, in a healthy organism the CSF gets completely renewed in 6–8 h (24), which would lead to a pretty fast peptide clearance, unless the process of peptide generation in the inflammation process is really intensive.

Among the KEGG and GO functional gene clusters observed in the CSF peptidomic data set, several clusters associated with the responses to various pathological processes (mainly bacterial and viral infections) were significantly over represented in the peptidogenic proteins in the AIDP samples. This might reflect an increase in the metabolism of these proteins during the course of AIDP, thereby suggesting the activation of related functions in the organism during the disease development. This is in accordance with the current hypothesis of infection-mediated GBS triggering. However, although the crucial role of *Campylobacter jejuni* (48) is well established for AMAN, the link between pathogenesis and different viral infections such as CMV (49) or EBV (50) in AIDP patients remain questionable. Herpes viruses—such as cytomegalovirus (CMV), Epstein-Barr virus (EBV) (13, 15), and the varicella-zoster virus (VZV)—have often been mentioned as possible GBS promoters (58), whereas the herpes simplex virus (HSV-1 and HSV-2) is rarely associated with GBS (59, 60). Our results again confirmed that none of the abovementioned viruses were strictly necessary for AIDP progression. At the same time, we found an elevated level of the overall viral infection in GBS patients. According to our results, 63% of AIDP and AMAN patients—in contrast to 20% of healthy individuals—were simultaneously seropositive for CMV, HHV-6, and HSV-1,2, thereby indicating that multiple simultaneous viral infections may contribute to triggering the GBS. This also indirectly correlated with the immune system activation in the AIDP which we observed as an increase in the antibody content as well as in the protein representation of the KEGG pathway “complement and coagulation cascades” both in the peptidome and the proteome.

The deposition of complement activation products on the outer surface of the Schwann cells of myelinated fibers was

shown earlier by Hafer-Macko *et al.* (61). Authors speculated that the complement was activated by antibodies binding to some epitopes on the outer surface of the Schwann cell and that the resulting complement activation initiated the vesiculation of myelin. Unfortunately, the authors did not succeed in showing this preliminary specific antibody reaction with the fiber surface probably because of technical reasons.

Another feature of the CSF from the AIDP patients was the presence of peptide fragments of several particular proteins which participate in the cellular adhesion: neurofascin, contactin-2, and NRCAM. The primary function of these proteins is to maintain the link between the Schwann cell membrane and the axonal membrane in the nodal, paranodal, and juxtapanodal regions of the myelinated peripheral nerve fibers. Currently, this is the first direct evidence of the AIDP-associated degradation of specific proteins participating in the arrangement of the myelin sheath around the nodes of Ranvier. Because at GBS there is no damage in the CNS, these CAM protein fragments must have come from the local inflammation sites in the DRGs. CAM fragments can be truncated from the nodes of Ranvier by the metalloproteinases produced by the activated macrophages, which are attracted to the areas of the DRG and spinal roots. Besides, because of the fast CSF renewal this process must be highly intensive and to continue throughout the disease progression.

Earlier, disorganization of the neurofascin and gliomedin aggregates was shown to precede demyelination in the experimental allergic neuritis induced in rat by immunization against peripheral myelin, thereby leading to the disruption of the nodal Na<sub>v</sub> ion channels and the subsequent conduction failure (62). Besides, antibodies to several proteins in the nodal area of the peripheral nerves - in particular, gliomedin, neurofascin, contactin (63, 64), and moesin (65) - were found in the blood stream of some AIDP patients. We also tested blood samples against the CAM proteins whose fragments we observed. A slightly enhanced titer of autoantibodies was found only against NRCAM and the difference was not as evident as in other classic autoimmune diseases with their autoantigens (53, 66). Thus, the degradation of the abovementioned proteins seems to have been associated with factors other than the autoreactive antibodies to those proteins. It is necessary to mention that in this work we did not aim at finding any potential AIDP specific autoantigens and the obtained results do not exclude their existence though other than the proteins tested. Furthermore, some researchers suggest the presence of such still unknown antigens for AIDP and suggest that their finding is a crucial key for defining pathogenic pathways in inflammatory neuropathies (67–69).

Another interesting finding in the AIDP CSF peptidome was the presence of protein fragments of the “sequestering of TGF- $\beta$  in the extracellular matrix” cluster, thus indicating the possibility of TGF- $\beta$  release. And TGF- $\beta$ -mediated regulation of the functional T-cell pool is one of the general mechanisms for the immune tolerance control (70). Another important func-

tion of TGF- $\beta$  is associated with the remyelination of the damaged peripheral nerves (71). TGF- $\beta$  undergoes conversion to its active form via several metalloproteinases, but plasmin (which releases TGF- $\beta$  from its complex with LTBP) and the proteins in the extracellular matrix also participate in this process (72, 73). The presence of fragments related to these proteins in the CSF of the AIDP patients may be regarded as an evidence of the activation of a TGF- $\beta$ -driven cascade. This conclusion is in line with the previously reported evidence that demonstrated a higher number of TGF- $\beta$ -secreting mononuclear cells (74) and an elevated concentration of the active form of TGF- $\beta$  in the blood and CSF samples obtained from GBS patients (75).

Because it is possible for blood proteins and locally generated peptides to enter the CSF through the inflammation sites, we also analyzed the spectra of cytokines in a search for some clues to the pathogenetic mechanism. Our results, as it might have been expected, did not show the cytokines associated with any general inflammation in the CNS in contrast to e.g. meningitis. The AIDP patients were characterized by an increased level of such mediators as IL-8, MCP-1, and GM-CSF anti-inflammatory cytokines - which are the main chemokines of monocyte activation and might attract monocytes and lymphocytes to the CSF through the BNB thus favoring the local inflammation. Besides, the AIDP CSF also showed elevated levels of IL-7 and IL-13. IL-7 is a neurotrophic factor, and its increase could be a protective reaction of the organism against injuries. IL-13 is the main anti-inflammatory cytokine, which suppresses macrophage activity by lowering the production of inflammatory cytokines and chemokines in response to IFN- $\gamma$ . It should also be stressed that both previously reported data and our current results point to the fact that the CSF-elevated cytokines do not simply penetrate the BBB or BNB in a passive manner, but rather percolate through the local sites of inflammation or locally originate in the CNS. The expression profile of the CSF cytokines for GBS patients differed from that of T-cells (TNF- $\alpha^{\text{high}}$ , IL-4, -5, -13, -17, -10, IFN- $\gamma$ ) and B-cells (IL-2 $^{\text{high}}$ , IL-4, -6, -12, TNF- $\alpha$ , IFN- $\gamma$ ), which are the main representatives of adaptive immunity. Finally, the cytokine profile of the CSF in the AIDP patients seemed to be rather heterogeneous and only indirectly associated with adaptive immune cells.

The development of AIDP is a low-probability and evidently multifactorial process. The classical generalized GBS model suggests the involvement of the autoimmunity and autoantibody production against some autoantigens on the surface of peripheral nerves. Such hypothesis is supported by the only well-established mechanism of GBS for its axonal forms which involves antibody production against the bacteria *C. jejuni* which in turn through the mechanism of molecular mimicry bind autoantigens in the PNS. However, despite all the efforts such AIDP-specific autoantigens remain undiscovered, whereas sporadic autoantibodies against different neurospecific proteins are being constantly reported (63, 76). We spec-

ulate that the local inflammation of a nerve fiber as a result of egress of neurotropic viruses superimposed with an activated state of the immune system as a result of a bacterial and/or viral infection (possibly simultaneous polyinfections) might lead to rapid expansion of inflammation along the peripheral nervous system. The clearance of initial pathogens initiates remyelination and natural mechanisms of immune suppression, thereby preventing further development of autoimmune responses in most cases. In such model an autoimmune factor might serve not as an initiator of the myelin sheath damaging but be a secondary process following neuroproteins leakage into the blood. And such spontaneous autoantibody production, which might depend on the individual features of the immune system, might further complicate the recovery and increase the overall damage level and severity of the disease. This hypothesis may partly explain the extremely aggressive development of AIDP with dynamics that basically exceeds the capabilities of the adaptive immunity.

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#### REFERENCES

- Rostami, A., and Sater, R. A. (1997) Guillain-Barré Syndrome. In: Rolak, L. A., and Yadollah, ed. *Neuroimmunology for the clinicians*, pp. 205–228, Butterworth-Heinemann, Boston
- Rostami, A. M. (1995) Guillain-Barre syndrome: clinical and immunological aspects. *Springer Sem. Immunopathol.* **17**, 29–42
- Hughes, R. A., and Cornblath, D. R. (2005) Guillain-Barre syndrome. *Lancet* **366**, 1653–1666
- Landry, O. (1859) Note sur la paralysie ascendante aigue. *Gaz. Hebd. Med. Chir.* **6**, 472–474, 486–488
- Guillain, G., Barre, J. A., and Strohl, A. (1916) Sur un syndrome de radiculo-nevrite avec hyperalbuminose du liquide cephalo-rachidien sans reaction cellulaire. Remarques sur les caracteres cliniques et graphique des reflexes tendineux. *Bull. et Mém. de la Soc. Méd. des Hôp. de Paris* **40**, 1462–1470
- Waksman, B. H., and Adams, R. D. (1955) Allergic neuritis: an experimental disease of rabbits induced by the injection of peripheral nervous tissue and adjuvants. *J. Exp. Med.* **102**, 213–236
- Asbury, A. K., Arnason, B. G., and Adams, R. D. (1969) The inflammatory lesion in idiopathic polyneuritis. Its role in pathogenesis. *Medicine* **48**, 173–215
- Constantinescu, C. S., Hilliard, B., Fujioka, T., Bhopale, M. K., Calida, D., and Rostami, A. M. (1998) Pathogenesis of neuroimmunologic diseases. Experimental models. *Immunol. Res.* **17**, 217–227
- Fujioka, T., Purev, E., Kremlev, S. G., Ventura, E. S., and Rostami, A. (2000) Flow cytometric analysis of infiltrating cells in the peripheral nerves in experimental allergic neuritis. *J. Neuroimmunol.* **108**, 181–191
- Kishore, A., Kanaujia, A., Nag, S., Rostami, A. M., Kenyon, L. C., Shindler, K. S., and Das Sarma, J. (2013) Different mechanisms of inflammation induced in virus and autoimmune-mediated models of multiple sclerosis in C57BL6 mice. *BioMed Res. Int.* 2013, 589048
- Sejvar, J. J., Baughman, A. L., Wise, M., and Morgan, O. W. (2011) Population incidence of Guillain-Barre syndrome: a systematic review and meta-analysis. *Neuroepidemiology* **36**, 123–133
- Cosi, V., and Versino, M. (2006) Guillain-Barre syndrome. *Neurol. Sci.* **27**, S47–S51
- Jacobs, B. C., Rothbarth, P. H., van der Meche, F. G., Herbrink, P.,

- Schmitz, P. I., de Klerk, M. A., and van Doorn, P. A. (1998) The spectrum of antecedent infections in Guillain-Barre syndrome: a case-control study. *Neurology* **51**, 1110–1115
14. Poropatich, K. O., Walker, C. L., and Black, R. E. (2010) Quantifying the association between *Campylobacter* infection and Guillain-Barre syndrome: a systematic review. *J. Health, Population, Nutrition* **28**, 545–552
  15. Orlikowski, D., Porcher, R., Sivadon-Tardy, V., Quincampoix, J. C., Raphael, J. C., Durand, M. C., Sharshar, T., Roussi, J., Caudie, C., Annane, D., Rozenberg, F., Leruez-Ville, M., Gaillard, J. L., and Gault, E. (2011) Guillain-Barre syndrome following primary cytomegalovirus infection: a prospective cohort study. *Clin. Infectious Dis.* **52**, 837–844
  16. Cao-Lormeau, V. M., Blake, A., Mons, S., Lastere, S., Roche, C., Vanhomwegen, J., Dub, T., Baudouin, L., Teissier, A., Larre, P., Vial, A. L., Decam, C., Choumet, V., Halstead, S. K., Willison, H. J., Musset, L., Manuguerra, J. C., Despres, P., Fournier, E., Mallet, H. P., Musso, D., Fontanet, A., Neil, J., and Ghawche, F. (2016) Guillain-Barre Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *Lancet* **387**, 1531–1539
  17. Yuki, N. (2007) Ganglioside mimicry and peripheral nerve disease. *Muscle Nerve* **35**, 691–711
  18. Zhang, H. L., Zheng, X. Y., and Zhu, J. (2013) Th1/Th2/Th17/Treg cytokines in Guillain-Barre syndrome and experimental autoimmune neuritis. *Cytokine Growth Factor Rev.* **24**, 443–453
  19. Vriesendorp, F. J., Mishu, B., Blaser, M. J., and Koski, C. L. (1993) Serum antibodies to GM1, GD1b, peripheral nerve myelin, and *Campylobacter jejuni* in patients with Guillain-Barre syndrome and controls: correlation and prognosis. *Anna. Neurol.* **34**, 130–135
  20. Kamakura, K., Kaida, K., Kusunoki, S., Miyamoto, N., Masaki, T., Nakamura, R., Motoyoshi, K., and Fukuda, J. (2005) Harmful effects of anti-GalNAc-GD1a antibodies and TNF-alpha on rat dorsal root ganglia. *J. Peripheral Nervous Syst.* **10**, 190–201
  21. Gabibov, A. G., Belogurov, A. A., Jr., Lomakin, Y. A., Zakharova, M. Y., Avakyan, M. E., Dubrovskaya, V. V., Smirnov, I. V., Ivanov, A. S., Molnar, A. A., Gurtsevitch, V. E., Diduk, S. V., Smirnova, K. V., Avalle, B., Sharanova, S. N., Tramontano, A., Friboulet, A., Boyko, A. N., Ponomarenko, N. A., and Tikunova, N. V. (2011) Combinatorial antibody library from multiple sclerosis patients reveals antibodies that cross-react with myelin basic protein and EBV antigen. *FASEB J.* **25**, 4211–4221
  22. Rosen, B. A. (2012) Guillain-Barre syndrome. *Pediatrics Rev.* **33**, 164–170; quiz 170–161
  23. Sindic, C. J., Van Antwerpen, M. P., and Goffette, S. (2001) The intrathecal humoral immune response: laboratory analysis and clinical relevance. *Clin. Chem. Lab. Med.* **39**, 333–340
  24. Zhang, J. (2007) Proteomics of human cerebrospinal fluid - the good, the bad, and the ugly. *Proteomics. Clin. Appl.* **1**, 805–819
  25. Jin, T., Hu, L. S., Chang, M., Wu, J., Winblad, B., and Zhu, J. (2007) Proteomic identification of potential protein markers in cerebrospinal fluid of GBS patients. *Eur. J. Neurol.* **14**, 563–568
  26. Lehmsiek, V., Sussmuth, S. D., Brettschneider, J., Tauscher, G., Felk, S., Gillardon, F., and Tumani, H. (2007) Proteome analysis of cerebrospinal fluid in Guillain-Barre syndrome (GBS). *J. Neuroimmunol.* **185**, 190–194
  27. D'Aguanno, S., Franciotta, D., Lupisella, S., Barassi, A., Pieragostino, D., Lugaresi, A., Centonze, D., D'Eril, G. M., Bernardini, S., Federici, G., and Urbani, A. (2010) Protein profiling of Guillain-Barre syndrome cerebrospinal fluid by two-dimensional electrophoresis and mass spectrometry. *Neuroscience Lett.* **485**, 49–54
  28. Yang, Y. R., Liu, S. L., Qin, Z. Y., Liu, F. J., Qin, Y. J., Bai, S. M., and Chen, Z. Y. (2008) Comparative proteomics analysis of cerebrospinal fluid of patients with Guillain-Barre syndrome. *Cell. Mol. Neurobiol.* **28**, 737–744
  29. Sejvar, J. J., Kohl, K. S., Gidudu, J., Amato, A., Bakshi, N., Baxter, R., Burwen, D. R., Cornblath, D. R., Cleerhout, J., Edwards, K. M., Heininger, U., Hughes, R., Khuri-Bulos, N., Korinthenberg, R., Law, B. J., Munro, U., Maltezou, H. C., Nell, P., Oleske, J., Sparks, R., Velentgas, P., Vermeer, P., and Witznitzer, M. (2011) Guillain-Barre syndrome and Fisher syndrome: case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* **29**, 599–612
  30. Hadden, R. D., Cornblath, D. R., Hughes, R. A., Zielasek, J., Hartung, H. P., Toyka, K. V., and Swan, A. V. (1998) Electrophysiological classification of Guillain-Barre syndrome: clinical associations and outcome. Plasma Exchange/Sandoglobulin Guillain-Barre Syndrome Trial Group. *Anna. Neurol.* **44**, 780–788
  31. Uncini, A., Zappasodi, F., and Notturmo, F. (2015) Electrodiagnosis of GBS subtypes by a single study: not yet the squaring of the circle. *J. Neurol. Neurosurgery, Psychiatry* **86**, 5–8
  32. McDonald, W. I., Compston, A., Edan, G., Goodkin, D., Hartung, H. P., Lublin, F. D., McFarland, H. F., Paty, D. W., Polman, C. H., Reingold, S. C., Sandberg-Wollheim, M., Sibley, W., Thompson, A., van den Noort, S., Weinschenker, B. Y., and Wolinsky, J. S. (2001) Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Anna. Neurol.* **50**, 121–127
  33. Giudicelli, V., Duroux, P., Ginestoux, C., Folch, G., Jabado-Michaloud, J., Chaume, D., and Lefranc, M. P. (2006) IMGT/LIGM-DB, the IMGT comprehensive database of immunoglobulin and T cell receptor nucleotide sequences. *Nucleic Acids Res.* **34**, D781–D784
  34. Zougman, A., Pilch, B., Podtelejnikov, A., Kiehnopf, M., Schnabel, C., Kumar, C., and Mann, M. (2008) Integrated analysis of the cerebrospinal fluid peptidome and proteome. *J. Proteome Res.* **7**, 386–399
  35. Holttta, M., Zetterberg, H., Mirgorodskaya, E., Mattsson, N., Blennow, K., and Gobom, J. (2012) Peptidome analysis of cerebrospinal fluid by LC-MALDI MS. *PLoS One* **7**, e42555
  36. Hyung, S. W., Piehowski, P. D., Moore, R. J., Orton, D. J., Schepmoes, A. A., Clauss, T. R., Chu, R. K., Fillmore, T. L., Brewer, H., Liu, T., Zhao, R., and Smith, R. D. (2014) Microscale depletion of high abundance proteins in human biofluids using IgY14 immunoaffinity resin: analysis of human plasma and cerebrospinal fluid. *Anal. Bioanal. Chem.* **406**, 7117–7125
  37. Vizcaino, J. A., Deutsch, E. W., Wang, R., Csordas, A., Reisinger, F., Rios, D., Dianes, J. A., Sun, Z., Farrah, T., Bandeira, N., Binz, P. A., Xenarios, I., Eisenacher, M., Mayer, G., Gatto, L., Campos, A., Chalkley, R. J., Kraus, H. J., Albar, J. P., Martinez-Bartolome, S., Apweiler, R., Omenn, G. S., Martens, L., Jones, A. R., and Hermjakob, H. (2014) ProteomeXchange provides globally coordinated proteomics data submission and dissemination. *Nat. Biotechnol.* **32**, 223–226
  38. Vizcaino, J. A., Cote, R. G., Csordas, A., Dianes, J. A., Fabregat, A., Foster, J. M., Griss, J., Alpi, E., Birim, M., Contell, J., O'Kelly, G., Schoenegger, A., Ovelleiro, D., Perez-Riverol, Y., Reisinger, F., Rios, D., Wang, R., and Hermjakob, H. (2013) The PRoteomics IDentifications (PRIDE) database and associated tools: status in 2013. *Nucleic Acids Res.* **41**, D1063–D1069
  39. Lomakin, Y. A., Zakharova, M. Y., Stepanov, A. V., Dronina, M. A., Smirnov, I. V., Bobik, T. V., Pyrkov, A. Y., Tikunova, N. V., Sharanova, S. N., Boitsov, V. M., Vyazmin, S. Y., Kabilov, M. R., Tupikin, A. E., Krasnov, A. N., Bykova, N. A., Medvedeva, Y. A., Fridman, M. V., Favorov, A. V., Ponomarenko, N. A., Dubina, M. V., Boyko, A. N., Vlassov, V. V., Belogurov, A. A., Jr., and Gabibov, A. G. (2014) Heavy-light chain interrelations of MS-associated immunoglobulins probed by deep sequencing and rational variation. *Mol. Immunol.* **62**, 305–314
  40. Micallef, L., and Rodgers, P. (2014) eulerAPE: drawing area-proportional 3-Venn diagrams using ellipses. *PLoS One* **9**, e101717
  41. Vucic, S., Kiernan, M. C., and Cornblath, D. R. (2009) Guillain-Barre syndrome: an update. *Clin. Neurosci.* **16**, 733–741
  42. Ropper, A. H., Wijdicks, E. F. M., and Truax, B. T. (1991) *Guillain-Barre syndrome*, F.A. Davis, Philadelphia
  43. Chiang, H. L., Lyu, R. K., Tseng, M. Y., Chang, K. H., Chang, H. S., Hsu, W. C., Kuo, H. C., Chu, C. C., Wu, Y. R., Ro, L. S., Huang, C. C., and Chen, C. M. (2009) Analyses of transthyretin concentration in the cerebrospinal fluid of patients with Guillain-Barre syndrome and other neurological disorders. *Clin. Chim. Acta* **405**, 143–147
  44. Zhang, H. L., Zhang, X. M., Mao, X. J., Deng, H., Li, H. F., Press, R., Fredrikson, S., and Zhu, J. (2012) Altered cerebrospinal fluid index of prealbumin, fibrinogen, and haptoglobin in patients with Guillain-Barre syndrome and chronic inflammatory demyelinating polyneuropathy. *Acta Neurol. Scandinavica* **125**, 129–135
  45. Brettschneider, J., Claus, A., Kassubek, J., and Tumani, H. (2005) Isolated blood-cerebrospinal fluid barrier dysfunction: prevalence and associated diseases. *J. Neurol.* **252**, 1067–1073
  46. Bremer, J., Baumann, F., Tiberi, C., Wessig, C., Fischer, H., Schwarz, P., Steele, A. D., Toyka, K. V., Nave, K. A., Weis, J., and Aguzzi, A. (2010) Axonal prion protein is required for peripheral myelin maintenance. *Nat. Neurosci.* **13**, 310–318
  47. Mallery, D. L., McEwan, W. A., Bidgood, S. R., Towers, G. J., Johnson, C. M., and James, L. C. (2010) Antibodies mediate intracellular immunity

- through tripartite motif-containing 21 (TRIM21). *Proc. Natl. Acad. Sci. USA* **107**, 19985–19990
48. Godschalk, P. C., Heikema, A. P., Gilbert, M., Komagamine, T., Ang, C. W., Glerum, J., Brochu, D., Li, J., Yuki, N., Jacobs, B. C., van Belkum, A., and Endtz, H. P. (2004) The crucial role of *Campylobacter jejuni* genes in anti-ganglioside antibody induction in Guillain-Barre syndrome. *J. Clin. Invest.* **114**, 1659–1665
  49. Steininger, C., Seiser, A., Gueler, N., Puchhammer-Stockl, E., Aberle, S. W., Stanek, G., and Popow-Kraupp, T. (2007) Primary cytomegalovirus infection in patients with Guillain-Barre syndrome. *J. Neuroimmunol.* **183**, 214–219
  50. Taheraghdam, A., Pourkhanjar, P., Talebi, M., Bonyadi, M., Pashapour, A., Sharifipour, E., and Rikhtegar, R. (2014) Correlations between cytomegalovirus, Epstein-Barr virus, anti-ganglioside antibodies, electrodiagnostic findings and functional status in Guillain-Barre syndrome. *Iranian J. Neurol.* **13**, 7–12
  51. Yuki, N., and Kuwabara, S. (2007) Axonal Guillain-Barre syndrome: carbohydrate mimicry and pathophysiology. *J. Peripheral Nervous Syst.* **12**, 238–249
  52. Ponomarenko, N. A., Durova, O. M., Vorobiev, I., Belogurov, A. A., Jr., Kurkova, I. N., Petrenko, A. G., Telegin, G. B., Suchkov, S. V., Kiselev, S. L., Lagarkova, M. A., Govorun, V. M., Serebryakova, M. V., Avasle, B., Tornatore, P., Karavanov, A., Morse, H. C., 3rd, Thomas, D., Friboulet, A., and Gabibov, A. G. (2006) Autoantibodies to myelin basic protein catalyze site-specific degradation of their antigen. *Proc. Natl. Acad. Sci. USA* **103**, 281–286
  53. Belogurov, A. A., Jr., Kurkova, I. N., Friboulet, A., Thomas, D., Misikov, V. K., Zakharova, M. Y., Suchkov, S. V., Kotov, S. V., Alehin, A. I., Avasle, B., Souslova, E. A., Morse, H. C., 3rd, Gabibov, A. G., and Ponomarenko, N. A. (2008) Recognition and degradation of myelin basic protein peptides by serum autoantibodies: novel biomarker for multiple sclerosis. *J. Immunol.* **180**, 1258–1267
  54. Jacobs, J. M., Macfarlane, R. M., and Cavanagh, J. B. (1976) Vascular leakage in the dorsal root ganglia of the rat, studied with horseradish peroxidase. *J. Neurol. Sci.* **29**, 95–107
  55. Prineas, J. W. (1981) Pathology of the Guillain-Barre syndrome. *Anna. Neurol.* **9**, 6–19
  56. Yang, M., Rainone, A., Shi, X. Q., Fournier, S., and Zhang, J. (2014) A new animal model of spontaneous autoimmune peripheral polyneuropathy: implications for Guillain-Barre syndrome. *Acta Neuropathol. Commun.* **2**, 5
  57. Mahboob, S., Mohamedali, A., Ahn, S. B., Schulz-Knappe, P., Nice, E., and Baker, M. S. (2015) Is isolation of comprehensive human plasma peptides an achievable quest? *J. Proteomics* **127**, 300–309
  58. Kang, J. H., Sheu, J. J., and Lin, H. C. (2010) Increased risk of Guillain-Barre Syndrome following recent herpes zoster: a population-based study across Taiwan. *Clin. Infectious Dis.* **51**, 525–530
  59. Kristensson, K., Lycke, E., and Sjostrand, J. (1971) Spread of herpes simplex virus in peripheral nerves. *Acta Neuropathol.* **17**, 44–53
  60. De Fine Olivarius, B., and Buhl, M. (1975) Herpes simplex virus and Guillain-Barre polyradiculitis. *Br. Med. J.* **1**, 192–193
  61. Hafer-Macko, C. E., Sheikh, K. A., Li, C. Y., Ho, T. W., Cornblath, D. R., McKhann, G. M., Asbury, A. K., and Griffin, J. W. (1996) Immune attack on the Schwann cell surface in acute inflammatory demyelinating polyneuropathy. *Anna. Neurol.* **39**, 625–635
  62. Lonigro, A., and Devaux, J. J. (2009) Disruption of neurofascin and gliomedin at nodes of Ranvier precedes demyelination in experimental allergic neuritis. *Brain* **132**, 260–273
  63. Devaux, J. J., Odaka, M., and Yuki, N. (2012) Nodal proteins are target antigens in Guillain-Barre syndrome. *J. Peripheral Nervous Syst.* **17**, 62–71
  64. Kawamura, N., Yamasaki, R., Yonekawa, T., Matsushita, T., Kusunoki, S., Nagayama, S., Fukuda, Y., Ogata, H., Matsuse, D., Murai, H., and Kira, J. (2013) Anti-neurofascin antibody in patients with combined central and peripheral demyelination. *Neurology* **81**, 714–722
  65. Sawai, S., Satoh, M., Mori, M., Misawa, S., Sogawa, K., Kazami, T., Ishibashi, M., Beppu, M., Shibuya, K., Ishige, T., Sekiguchi, Y., Noda, K., Sato, K., Matsushita, K., Kodera, Y., Nomura, F., and Kuwabara, S. (2014) Moesin is a possible target molecule for cytomegalovirus-related Guillain-Barre syndrome. *Neurology* **83**, 113–117
  66. Arbuckle, M. R., McClain, M. T., Rubertone, M. V., Scofield, R. H., Dennis, G. J., James, J. A., and Harley, J. B. (2003) Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl. J. Med.* **349**, 1526–1533
  67. Hughes, R. A., and Willison, H. J. (2012) Neurofascin antibodies in inflammatory neuropathy: how many needles make a haystack? *Neurology* **79**, 2224–2225
  68. Lim, J. P., Devaux, J., and Yuki, N. (2014) Peripheral nerve proteins as potential autoantigens in acute and chronic inflammatory demyelinating polyneuropathies. *Autoimmunity Rev.* **13**, 1070–1078
  69. Willison, H., and Scherer, S. S. (2014) Ranvier revisited: novel nodal antigens stimulate interest in GBS pathogenesis. *Neurology* **83**, 106–108
  70. Oh, S. A., and Li, M. O. (2013) TGF-beta: guardian of T cell function. *J. Immunol.* **191**, 3973–3979
  71. Einheber, S., Hannocks, M. J., Metz, C. N., Rifkin, D. B., and Salzer, J. L. (1995) Transforming growth factor-beta 1 regulates axon/Schwann cell interactions. *J. Cell Biol.* **129**, 443–458
  72. Lyons, R. M., Gentry, L. E., Purchio, A. F., and Moses, H. L. (1990) Mechanism of activation of latent recombinant transforming growth factor beta 1 by plasmin. *J. Cell Biol.* **110**, 1361–1367
  73. Taylor, A. W. (2009) Review of the activation of TGF-beta in immunity. *J. Leukocyte Biol.* **85**, 29–33
  74. Dahle, C., Kvarnstrom, M., Ekerfelt, C., Samuelsson, M., and Emerudh, J. (2003) Elevated number of cells secreting transforming growth factor beta in Guillain-Barre syndrome. *APMIS* **111**, 1095–1104
  75. Ossege, L. M., Sindern, E., Voss, B., and Malin, J. P. (2000) Expression of TNFalpha and TGFbeta1 in Guillain-Barre syndrome: correlation of a low TNFalpha-TGFbeta1-mRNA ratio with good recovery and signs for immunoregulation within the cerebrospinal fluid compartment. *Eur. J. Neurol.* **7**, 17–25
  76. Kwa, M. S., van Schaik, I. N., De Jonge, R. R., Brand, A., Kalaydjieva, L., van Belzen, N., Vermeulen, M., and Baas, F. (2003) Autoimmunoreactivity to Schwann cells in patients with inflammatory neuropathies. *Brain* **126**, 361–375