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Autoimmune Glial Fibrillary Acidic Protein Astrocytopathy
A Novel Meningoencephalomyelitis

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IMPORTANCE A novel astrocytic autoantibody has been identified as a biomarker of a relapsing autoimmune meningoencephalomyelitis that is immunotherapy responsive. Seropositivity distinguishes autoimmune glial fibrillary acidic protein (GFAP) meningoencephalomyelitis from disorders commonly considered in the differential diagnosis.

OBJECTIVE To describe a novel IgG autoantibody found in serum or cerebrospinal fluid that is specific for a cytosolic intermediate filament protein of astrocytes.

DESIGN, SETTING, AND PARTICIPANTS Retrospective review of the medical records of seropositive patients identified in the Mayo Clinic Neuroimmunology Laboratory from October 15, 1998, to April 1, 2016, in blinded comprehensive serologic evaluation for autoantibody profiles to aid the diagnosis of neurologic autoimmunity (and predict cancer likelihood).

MAIN OUTCOMES AND MEASURES Frequency and definition of novel autoantibody, the autoantigen's immunochemical identification, clinical and magnetic resonance imaging correlations of the autoantibody, and immunotherapy responsiveness.

RESULTS Of 103 patients whose medical records were available for review, the 16 initial patients identified as seropositive were the subject of this study. Median age at neurologic symptom onset was 42 years (range, 21-73 years); there was no sex predominance. The novel neural autoantibody, which we discovered to be GFAP-specific, is disease spectrum restricted but not rare (frequency equivalent to Purkinje cell antibody type 1 [anti-Yo]). Its filamentous pial, subventricular, and perivascular immunostaining pattern on mouse tissue resembles the characteristic magnetic resonance imaging findings of linear perivascular enhancement in patients. Prominent clinical manifestations are headache, subacute encephalopathy, optic papillitis, inflammatory myelitis, postural tremor, and cerebellar ataxia. Cerebrospinal fluid was inflammatory in 13 of 14 patients (93%) with data available. Neoplasia was diagnosed within 3 years of neurologic onset in 6 of 16 patients (38%): prostate and gastroesophageal adenocarcinomas, myeloma, melanoma, colonic carcinoid, parotid pleomorphic adenoma, and teratoma. Neurologic improvement followed treatment with high-dose corticosteroids, with a tendency of patients to relapse without long-term immunosuppression.

CONCLUSIONS AND RELEVANCE Glial fibrillary acidic protein–specific IgG identifies a distinctive, corticosteroid-responsive, sometimes paraneoplastic autoimmune meningoencephalomyelitis. It has a lethal canine equivalent: necrotizing meningoencephalitis. Expression of GFAP has been reported in some of the tumor types identified in paraneoplastic cases. Glial fibrillary acidic protein peptide–specific cytotoxic CD8^{+} T cells are implicated as effectors in a transgenic mouse model of autoimmune GFAP meningoencephalitis.

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Neural antigen-specific autoimmune disorders are immunotherapy responsive and affect all nervous system levels.[1-3] Subacute or insidious symptom onset raises suspicion for an infectious, degenerative, demyelinating, neoplastic or vascular disorder. Detection in serum or cerebrospinal fluid (CSF) of neuronal, glial, or skeletal muscle-specific IgG aids diagnosis and guides appropriate therapeutic options. Paraneoplastic cases reflect immune responses incited by onconeural antigens in an occult systemic cancer. Informative autoantibody profiles predict high cancer probability[4,5] and yield immunopathogenic insights. The Mayo Clinic Neuroimmunology Laboratory's algorithm for neural-specific IgG detection incorporates a mouse tissue-based indirect immunofluorescence assay that, in 2% of evaluated cases, reveals clinically pertinent autoantibodies, some molecularly uncharacterized. We describe a novel autoantibody that yielded an astrocyte-restricted staining pattern.

Key Points

Question Can a serologic biomarker aid the diagnosis of autoimmune meningoencephalomyelitis?

Findings A novel astrocyte-specific IgG autoantibody discovered in the cerebrospinal fluid and serum of 103 patients undergoing testing for potential autoimmune neurologic disorders in a high-volume service laboratory was found in review of medical records to be associated with a disabling and relapsing corticosteroid-responsive meningoencephalitis, with or without myelitis. Glial fibrillary acidic protein (GFAP) was identified as the antigen, and one-third of cases were paraneoplastic.

Meaning As a disease biomarker, GFAP-specific IgG unifies a spectrum of treatable autoimmune meningoencephalomyelitis, a new class of autoimmune astrocytopathy that is presumably mediated by GFAP peptide–specific cytotoxic CD8+ T cells.

Methods

Study Population

Serum specimens used to characterize the novel autoantibody were representative of seropositive cases (134 to date) identified in a blinded service laboratory evaluation of more than 100 000 patients suspected clinically of having an autoimmune neurologic disorder. Control specimens included 455 serum specimens (mouse tissue-based immunofluorescence assay: 173 healthy Olmsted County Minnesota residents; glial fibrillary acidic protein [GFAP]-specific cell-based assays: 135 healthy Mayo Clinic Biobank participants [100 adults; 35 children], 20 patients with multiple sclerosis, 57 patients with aquaporin 4 [AQP4]-specific IgG–seropositive neuromyelitis optica spectrum disorder, 35 patients with systemic lupus erythematosus or Sjögren syndrome, and 35 patients with hypergammaglobulinemia) and 49 CSF specimens (GFAP-specific cell-based assays: 26 normal-pressure hydrocephalus [adults] and 23 miscellaneous disorders [children]). This report describes the autoantibody characteristics, antigen identity, and clinical synopsis of neurologic, oncologic, and radiologic findings, companion autoantibodies, and immunotherapy responses for the patients initially identified as seropositive. The study was performed from October 15, 1998, to April 1, 2016. The Mayo Clinic Institutional Review Board approved the study. Patients were identified in the Mayo Clinic Neuroimmunology Laboratory database. Records indicated research consent (Mayo Clinic patients) or physicians had obtained verbal consent (non–Mayo Clinic patients). Data were not deidentified but were stored in a password-protected database by 2 of us (B.F. and A.M.).

Immunohistochemical Assays

Screening used 4-μm cryosections of adult mouse cerebellum-midbrain-cerebral-cortex-hippocampus, kidney, and stomach.[6] Research analyses used juvenile rat spinal cord sections. After permeabilization (1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, 4 minutes), fixation (10% formalin, 4 minutes), and blocking (normal goat or swine serum, 10% in phosphate-buffered saline, 1 hour), we applied patient serum (preabsorbed with bovine liver powder, 1:120 dilution), CSF

(nonabsorbed, 50% dilution), or commercial polyclonal IgG antibodies: rabbit pan-GFAP (1:5000, Z 0334; Dako), GFAP-δ (1:500, PA1-06702; Pierce Biotechnology), or GFAP-ε (1:100, ab28926, ab93251; Abcam) or goat GFAP-α-specific (C-19) (1:100, sc-6170; Santa Cruz Biotechnology Inc). After 40 minutes and phosphate-buffered saline wash, we applied a secondary antibody (species-specific anti-IgG, fluorescein isothiocyanate, or tetramethylrhodamine conjugated, 35 minutes; Southern Biotechnology Associates Inc) and glass coverslips to washed sections using ProLong Gold antifade mounting medium (containing 4′,6-diamidino-2-phenylindole; Molecular Probes). Fluorescence images were captured using Axiovision software (Carl Zeiss Inc). Specimens that yielded positive results were titrated (doubling dilutions) to determine the autoantibody detection end point. For dual staining, we applied patient serum and rabbit monoclonal intermediate filament-specific IgG (eg, 1:200, vimentin [ab92547; Abcam] or desmin [ab32362; Abcam]) and secondary antibodies (1:100, tetramethylrhodamine-conjugated goat anti-rabbit IgG and donkey anti-human IgG; Jackson ImmunoResearch Laboratories). Confocal images were captured using a microscope (63 × or 40 × water immersion lens, LSM710; Carl Zeiss Inc).

Cultured Cells

HEK293 cell lines stably transfected with plasmids encoding GFAP homo sapiens transcript variant 1 (RG 204548, pCMV6-AC-GFAP-α-GFP) and variant 2 (RG225707, pCMV6-AC-GFAP-δ/ε-GFP, OriGene Inc) were selected in G418 (0.8 g/mL; Gibco BRL). Human glioblastoma multiforme (GBM) cells (serially xenografted in athymic nude mice) were provided by Jann Sarkaria, MD (Mayo Clinic).[7]

Cell-Based Immunofluorescence Assays

Cells fixed (4% paraformaldehyde, 15 minutes) and permeabilized (0.2% Triton X-100, 10 minutes) were held overnight at 4°C with patient serum (1:10 dilution), CSF (undiluted), rabbit pan-GFAP-specific IgG (1:5000), rabbit GFAP-ε-specific IgG (1:100), or goat GFAP-α-specific IgG (C-19, 1:20). After phosphate-buffered saline wash and incubation with secondary antibodies (1:200), we captured images by confocal microscopy (63 × or 40 × water immersion lens, LSM710; Carl Zeiss Inc).

Antigen Identification

Adult rat spinal cord and GBM cells were extracted in 150-mmol/L sodium chloride, 10-mmol/L sodium phosphate, and 2-mmol/L EDTA (pH 7.2), containing 1% Triton X-100, 0.1% sodium dodecyl sulfate, and protease inhibitors (Complete; Roche Pharmaceuticals). Lysate clarified by centrifugation (400g, 10 minutes) was sequentially centrifuged for 30 minutes (4000g, 8000g, 100 000g, and 300 000g). Reduced and denatured supernates and pellets of each fraction were separated by gel electrophoresis (10% polyacrylamide), transferred to nitrocellulose, and probed with patient serum or CSF (molecular weight standards, 161-0374; Bio-Rad Laboratories Inc). To determine molecular identity, we solubilized the most informative fraction in second-dimension electrophoresis sample buffer, loaded it onto 13-cm immobilized pH gradient (Immobiline DryStrip, GE Healthcare; pH 4-7), and applied 3500 V (final voltage) for 20 hours. Second-dimension electrophoresis was performed on 10% polyacrylamide gel. After nitrocellulose membrane transfer (0.45 µm; Bio-Rad Laboratories), separated proteins were visualized by silver staining and autoradiography (Western blot). Peptides were identified (Mascot search algorithm) in excised immunoreactive spots analyzed by in-gel digest and tandem mass spectrometry.

Western Blot

Stably transfected and nontransfected HEK293 cell lysates (in a solution of 50-mmol/L Tris hydrochloride, pH 7.5, 150-mmol/L sodium chloride, and 2% Triton X-100) were clarified by centrifugation (1000g, 5 minutes), electrophoresed in 10% polyacrylamide gel, transferred electrophoretically to nitrocellulose membrane, blocked in buffer (in a solution of 20-mmol/L Tris, pH 7.6, 137-mmol/L sodium chloride, 0.1% Tween-20) that contained 10% powdered milk, then probed 1 hour with IgG specific for GFAP-α (1:50), GFAP-ε/δ (1:100), pan-GFAP (1:10 000), actin (1:2000), patient serum (1:100), patient CSF (1:10), or healthy control serum and CSF. After three 5-minute washes (20-mmol/L Tris, pH 7.6, 137-mmol/L sodium chloride, 0.1% Tween-20), blots were incubated for 30 minutes with horseradish peroxidase–conjugated goat anti-rabbit IgG, swine anti-goat IgG, or goat anti-human IgG (1:2000). After washing, bound IgG was detected autoradiographically by enhanced chemiluminescence (SuperSignal West Pico Luminol/Enhancer; Thermo Fisher Scientific).

Results

Astrocytic Autoantibody Characterization

The 16 patients initially identified with this autoantibody had a unifying diagnosis of meningoencephalomyelitis that resembled earlier described nonvasculitic autoimmune inflammatory meningoencephalitis.[8,9] These 16 patients are the subject of this study. Median age at neurologic symptom onset was 42 years (range, 21-73 years); there was no sex predominance.

IgG in all 16 patients intensely stained cytoplasmic filaments in histologically restricted astrocyte populations. None of 173 Olmsted County healthy control serum specimens yielded this pattern. Apart from 87 subsequently identified
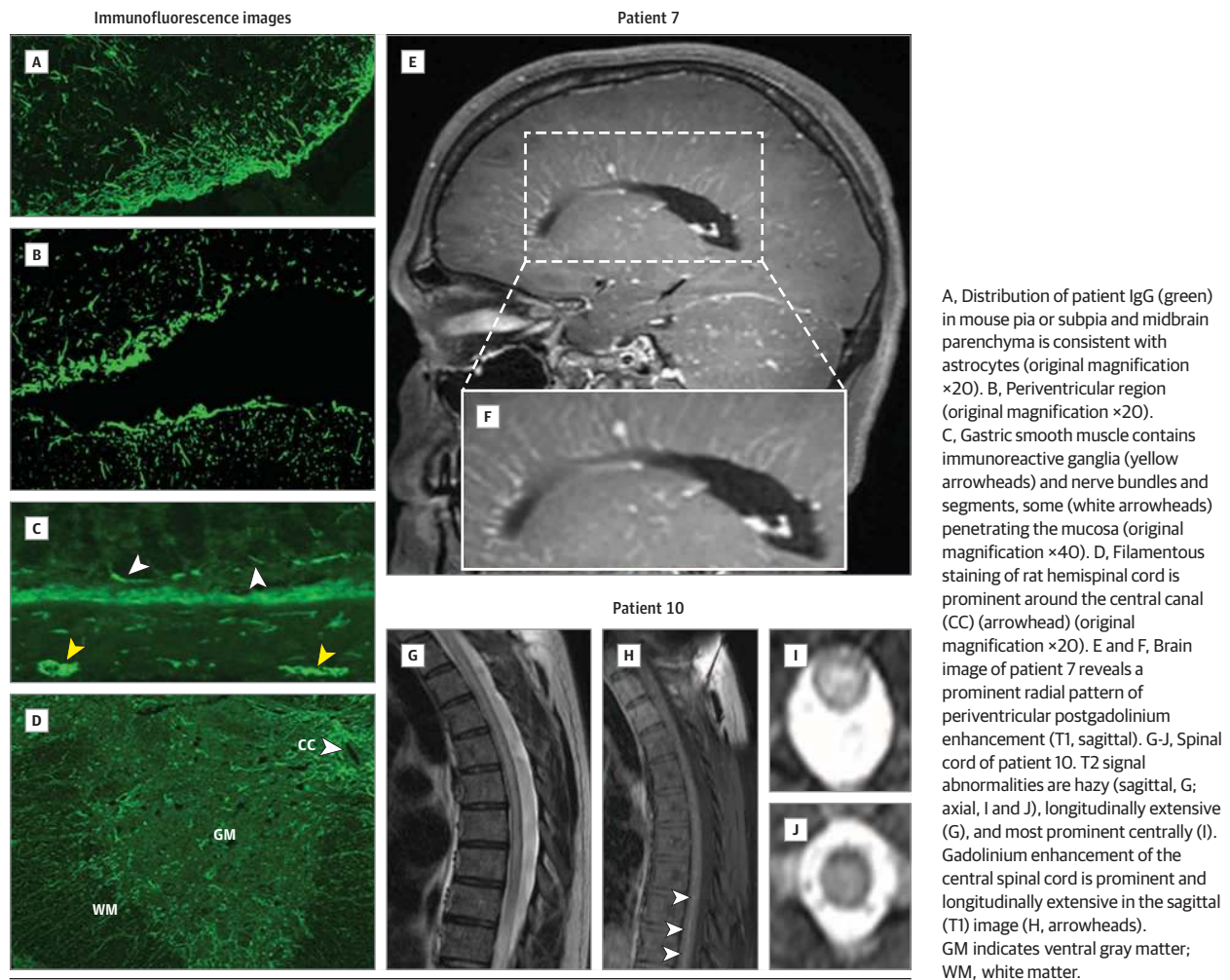
seropositive patients (eFigure 1 in the Supplement), this pattern was not yielded by any serum or CSF specimen among more than 100 000 patients with miscellaneous neurologic disorders tested by service tissue-based immunofluorescence assay. Immunostaining in mouse brain was confined to pia, subpia, and midbrain foci (**Figure 1**A); periventricular region (Figure 1B); and rostral migratory stream (eFigure 2 in the Supplement). Enteric ganglia and nerves with mucosa-penetrating filaments were prominent immunoreactive elements in the periphery (Figure 1C); renal nerve elements were nonimmunoreactive. In spinal cord, immunoreactive filaments were prominent around the central canal and in gray matter, radiating to pia (Figure 1D).

Neurologic Correlations

The **Table** summarizes the 16 patients' clinical and laboratory findings. Evaluations were not conducted uniformly, and no alternative diagnoses were established (infectious, granulomatous, inflammatory demyelinating, lymphomatous, carcinomatous, or vasculitic). The most common clinical presentation was disabling corticosteroid-responsive meningoencephalitis or encephalitis, with or without myelitis. Fourteen patients had meningeal and encephalitic symptoms; seven additionally had myelitic symptoms, 8 had vision changes, and 2 had isolated meningeal or encephalitic symptoms. Subacute headache was the most common symptom (13 patients). Prominent clinical findings were optic disc edema without increased intracranial pressure (optic papillitis in 7 patients), myelopathy, tremor, ataxia, progressive cognitive impairment, autonomic instability, and psychiatric disturbance. No patient had seizures. Continuing retrospective history review for subsequent GFAP-specific IgG–seropositive patients confirms the association with central nervous system (CNS) inflammation (to date 92% of 103 cases) (eFigure 1 in the Supplement); detailed clinical, radiologic, serologic, and CSF findings; responses to treatments; and outcomes will be the subject of a future article. Eight of 103 patients (8%) had a peripheral nervous system disorder (neuropathy, dysautonomia, or myasthenia gravis).

Cranial or spinal magnetic resonance imaging (MRI) (available for 12 of 16 patients [75%]) revealed diffuse T2 abnormalities in periventricular white matter (9 of 12 [75%]) (Table); 6 patients (50%) had prominent linear perivascular enhancement oriented radially to the ventricles, and 4 (33%) had leptomeningeal enhancement. Spinal MRI revealed longitudinally extensive T2 hyperintensity (5 of 7 patients [71%] with myelopathy) or produced normal results (myelitic symptoms in 2 of 7 patients [29%]). Figure 1E-I shows, for 2 patients, resemblances of MRI enhancement patterns (cranial and spinal, respectively) to the immunohistochemical staining patterns of patient IgG on meningeal and parenchymal elements in rodent brain and spinal cord (Figures 1B and D and eFigure 3 in the Supplement). The CSF was inflammatory in 13 of 14 patients with available data: leukocytes, 4 to 500/µL (median, 121/µL; reference range, ≤5/µL [to convert to ×10⁹/L, multiply by 0.001]; lymphocytes, >80%); and protein, 0.06 to 0.20 g/dL (median, 0.11 g/dL; reference range, ≤0.04 g/dL [to convert to grams per liter, multiply by 10]). Supernumerary oligoclonal bands were found in 5 patients and elevated IgG index

Figure 1. Immunofluorescence Pattern of Patient IgG Bound to Rodent Central Nervous System Tissues in Part
Resembles Brain and Spinal Cord Magnetic Resonance Imaging Patterns of Patients With Autoimmune Glial
Fibrillary Acidic Protein Meningoencephalomyelitis

A, Distribution of patient IgG (green) in mouse pia or subpia and midbrain parenchyma is consistent with astrocytes (original magnification ×20). B, Periventricular region (original magnification ×20). C, Gastric smooth muscle contains immunoreactive ganglia (yellow arrowheads) and nerve bundles and segments, some (white arrowheads) penetrating the mucosa (original magnification ×40). D, Filamentous staining of rat hemispinal cord is prominent around the central canal (CC) (arrowhead) (original magnification ×20). E and F, Brain image of patient 7 reveals a prominent radial pattern of periventricular postgadolinium enhancement (T1, sagittal). G-J, Spinal cord of patient 10. T2 signal abnormalities are hazy (sagittal, G; axial, I and J), longitudinally extensive (G), and most prominent centrally (I). Gadolinium enhancement of the central spinal cord is prominent and longitudinally extensive in the sagittal (T1) image (H, arrowheads). GM indicates ventral gray matter; WM, white matter.

in 3 patients. The CSF opening pressure was elevated (29.8 cm H_2O) in 1 of 8 recorded cases (13%). Available clinical, radiologic, and CSF findings classified the 16 patients as follows: meningoencephalitis, 6; meningoencephalomyelitis, 5; encephalomyelitis, 2; encephalitis, 2; and meningitis, 1 (Table).

Coexisting Disorders

Seven patients had 1 or more autoimmune findings (Table): glutamic acid decarboxylase 65-kDa isoform antibody in 3 patients (43%) (1 had type 1 diabetes), thyroperoxidase-specific IgG in 3 patients (43%) (1 had Graves thyroiditis), P/Q-type voltage-gated calcium channel antibody in 2 patients (29%) (1 had interstitial pneumonitis and myositis and 1 had prostate adenocarcinoma), N-methyl-D-aspartate receptor (NMDAR)–specific IgG in the CSF in 2 patients (29%) (1 paired serum specimen was also positive; both patients had meningoencephalitis, 1 had teratoma, and neither had classic autoimmune NMDAR encephalitis), and antinuclear antibody in 1 patient (14%). Miscellaneous immunopathies included polyclonal hypergammaglobulinemia and IgA deficiency.

Six patients had documented cancer, past or current (7 neoplasms, 5 after neurologic symptom onset and 2 before): 2 adenocarcinomas (prostate, gastroesophageal coexisting with myeloma), metastatic melanoma, colonic carcinoid, parotid pleomorphic adenoma (mixed tumor), and teratoma. The median interval from neurologic symptom onset to cancer diagnosis was 3 months (range, −24 to +36 months).

Eleven patients of 13 with treatment information (85%) received immunotherapy; all responded favorably to initial intravenous high-dose corticosteroid treatment, but 7 (64%) relapsed during dose tapering. No relapse occurred in 6 patients (55%) who received long-term immunosuppression (mycophenolate, 5; azathioprine, 1).

Autoantigen Identification
Immunohistochemical Analysis

We investigated intermediate filament antigens. Desmin immunoreactivity colocalized with patient IgG in pia and subpia (eFigure 4 in the Supplement); divergence in gut smooth muscle (patient IgG nonreactive) lessened the likelihood of

Table. Demographic, Clinical, Imaging, Serum, and CSF Findings in 16 GFAP-Specific IgG–Positive Patients[a]

Sex/Age at Onset, y	Diagnosis	Monophasic or Relapsing	Presenting Symptoms	MRI Findings	CSF Protein, g/L; White Blood Cell Count, /μL; No. of Unique Oligoclonal Bands; IgG Index	Coexisting Autoimmune Disease/ Autoantibodies (Value)	Cancer Detected	Immunotherapy Response	GFAP-Specific IgG Titer/Isoform Serum	CSF
F/31	Meningoencephalitis	Monophasic	Headache, weight loss, cognitive change, hemiparesis, vomiting, abnormal movements	Brain: diffuse leptomeningeal T2 hyperintensities and postgadolinium enhancement	0.94; 144 (99% lymphocytes); unknown; 1.08	No	No	No immunotherapy	491 520/α + ε	NA
F/43	Meningoencephalitis	Unknown	Altered mental status, hallucinations	Brain: diffuse gyral and leptomeningeal enhancement	0.80; 50 (80% lymphocytes); unknown; unknown	No	No	Unknown	61 440/α + ε	NA
F/27	Meningoencephalitis	Monophasic	Headache, hallucinations, obtundation	NA	Unknown; 500 (80% lymphocytes); unknown; unknown.	No		Improved	15 360/α	128/α + ε
M/73	Encephalitis[b]	Relapsing	Subacute onset lethargy, weight loss, confusion, imbalance, depression; viral encephalitis suspected; subsequent painless bilateral vision loss	Brain: diffuse T2 hyperintensities, periventricular white matter	Unknown; 4 (93% lymphocytes); unknown; unknown	Arthritis/VGCC-P/Q (0.04-nmol/L serum); thyroglobulin	Prostate adenocarcinoma	No immunotherapy	15 360/α	NA
F/21	Meningoencephalitis[b]	Relapsing	Headache, behavioral changes, delirium, paranoia, progressive imbalance, vision loss	Brain: cerebellar leptomeningeal T2 hyperintensities and postgadolinium enhancement.	0.11; 308 (96% lymphocytes); unknown; normal	NMDAR IgG (CSF positive)	No	Improved	3840/α + ε	256/NA
F/65	Meningoencephalomyelitis	Relapsing	Headache, dysphagia, tremor, meningismus, weight loss, limb weakness	NA	Elevated; elevated; unknown; unknown	Diabetes	PET: hypermetabolic uptake left hepatic lobe	Improved	3840/NA	NA
F/29	Meningoencephalomyelitis[b]	Relapsing	Headaches, photophobia, reduced taste/olfaction, vision loss, tremor, left lateral thigh numb	Brain and upper cord: diffuse perivascular and leptomeningeal enhancement, some nodular	0.19; 77 (95% lymphocytes); 8; 1.29	Graves thyroiditis/GAD65 (4.16-nmol/L serum); thyroperoxidase	Parotid pleomorphic adenoma	Improved	1920/Negative	512/α + ε
M/53	Encephalomyelitis	Monophasic	Headache, tremor, jerking limbs, palpitations, flushing, presyncopal sensation, blurred vision (due to left cranial nerve VI palsy); subsequent tremulousness, imbalance, urine retention, weight loss, diplopia, unstable gait, cognitive decline, night mares	Brain: diffuse radial periventricular T2 hyperintensities and postgadolinium enhancement (perivascular); cord: T2 abnormalities	0.20; 90 (99% lymphocytes); 6; normal	Interstitial pneumonia; nonspecific myositis/VGCC-P/Q (0.03-nmol/L serum)	No	Improved	1920/α	64/α + ε
F/43	Encephalitis	Relapsing	Headache, vomiting, weight loss, movement disorder, constipation, postural light-headedness, dry mouth	Brain: diffuse T2 hyperintensities in the left occipital and parietal lobes; perivascular enhancement, linear and nodular; cord: extensive T2 hyperintensities, cervical and thoracic	0.17; 148 (90% lymphocytes); 5; unknown	GAD65 (0.04-nmol/L serum); thyroperoxidase	No	Improved	1920/α + ε	256/α + ε

Table. Demographic, Clinical, Imaging, Serum, and CSF Findings in 16 GFAP-Specific IgG–Positive Patients[a] (continued)
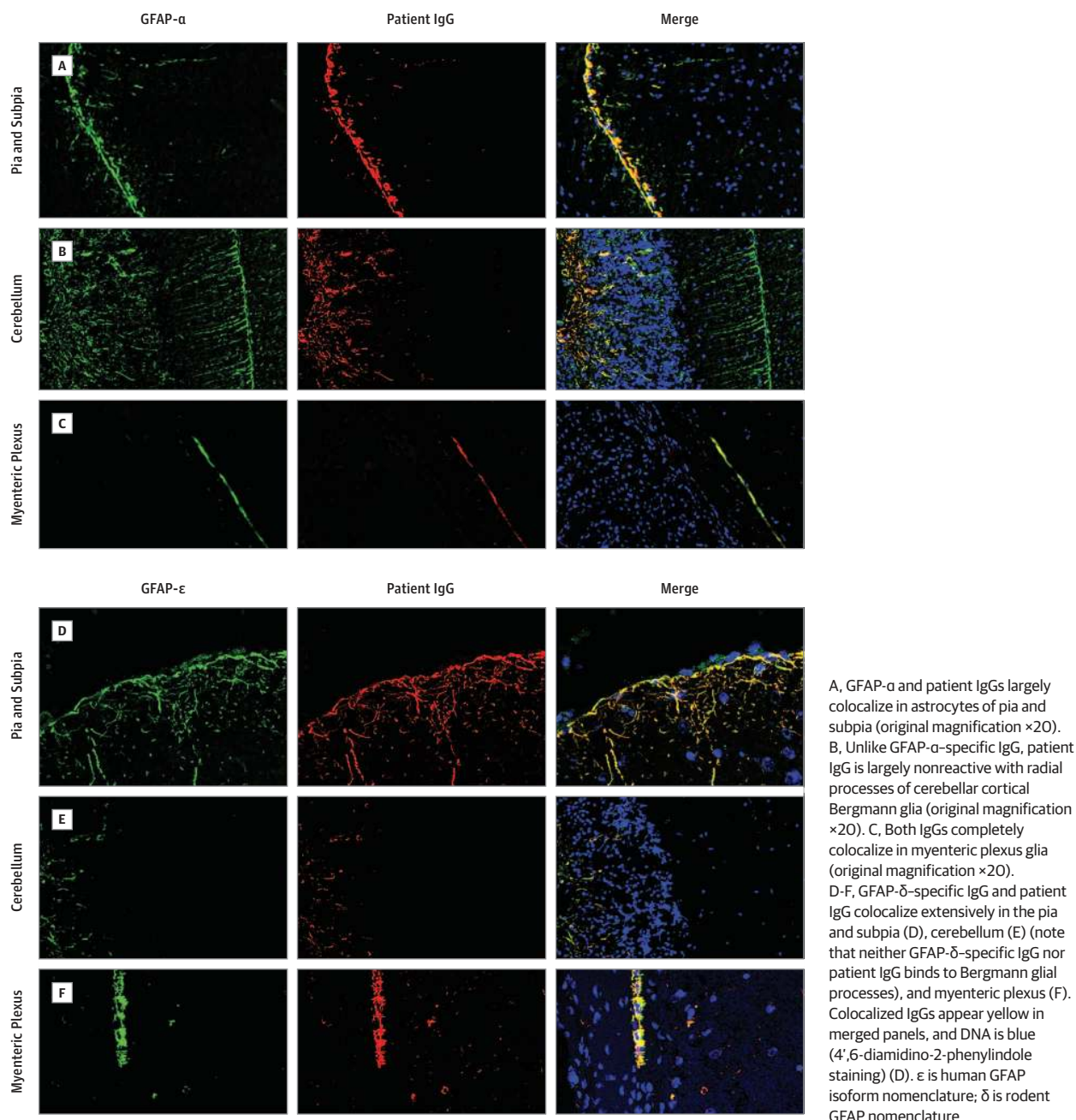
Sex/Age at Onset, y	Diagnosis	Monophasic or Relapsing	Presenting Symptoms	MRI Findings	CSF Protein, g/L; White Blood Cell Count, /μL; No. of Unique Oligoclonal Bands; IgG Index	Coexisting Autoimmune Disease/ Autoantibodies (Value)	Cancer Detected	Immunotherapy Response	GFAP-Specific IgG Titer/Isoform Serum	CSF
M/32	Encephalomyelitis[b]	Relapsing	Headache, subacute blurred vision, polyuria and polydipsia, weight loss; viral encephalitis suspected; fatigue, intractable insomnia, depression; progressed to gait disorder, urine retention, constipation, emotional lability, poor memory, confusion	Brain: diffuse hemispheric and pontine radial periventricular T2 hyperintensities, perivascular postgadolinium enhancement; cord: extensive T2 hyperintensities cervical and thoracic with parenchymal enhancement	0.79; 58 (88% lymphocytes); 2; 1.23	Diabetes type 1/GAD65 (0.24-nmol/L serum)	No	Improved	1920/Negative	NA
M/37	Meningoenceph-alomyelitis[b]	Relapsing	Headache, vision changes, tremor, imbalance, lightheaded; cognitive changes, weakness, sensory loss, erectile dysfunction	Brain and cord: diffuse brain and upper cervical T2 abnormalities; perivascular brain enhancement, leptomeningeal cord enhancement	0.11; 185 (97% lymphocytes); 5; unknown	No	No	Improved	480/α + ε	NA
M/51	Meningoenceph-alomyelitis[b]	Relapsing	Headache, subacute tremor, weight loss, gastrointestinal symptoms, fatigue, blurred vision, malaise, emotional lability, hyperactive startle, cognitive change	Brain and cord: diffuse brain and lower thoracic cord T2 abnormalities, perivascular brain enhancement, leptomeningeal cord enhancement	0.10; 121 (98% lymphocytes); none; normal	No	Colonic carcinoid	Improved	120/Negative	256/α ε
M/40	Meningo-encephalitis	Unknown	Headache; no other details	NA	NA	No	Metastatic melanoma	Unknown	NA	256/α + ε
F/25	Meningoenceph-alomyelitis	Monophasic	Flu-like illness, myelitis, coma; no other details	NA	NA	NMDAR-specific IgG (CSF positive)	Teratoma	Unknown	NA	8192/α + ε
M/21	Chronic meningitis[b]	Monophasic	Headache, weight loss, vision changes, nausea, aural fluttering sound, abdominal pain, orthostatic dizziness	Brain: small areas of nonenhancing T2 hyperintensity in white matter and caudate head	0.64; 26 (97% lymphocytes); 6; 0.90	Diabetes type 1, alopecia universalis/ thyroperoxidase, SCL70, ANA, cold agglutinin, polyclonal gammopathy	No	Improved	NA	256/α + ε
M/61	Meningo-encephalitis[b]	Monophasic	Headache, fever, confusion	Small areas of T2 hyperintensity hemispheric white matter; enhancement (<1 cm) right temporal lobe	0.15; 190 (98% lymphocytes); normal; normal	No	Multiple myeloma	Improved	NA	512/α + ε

Abbreviations: ANA, antinuclear antibody; CSF, cerebrospinal fluid; GAD65, 65-kDa isoform of glutamic acid decarboxylase; GFAP, glial fibrillary acidic protein; MRI, magnetic resonance images; NA, not available; NMDAR, N-methyl-D-aspartate receptor; PET, positron emission tomography; SCL70, 70-kDa immunoreactive fragment extractable from topisomerase 1 antigen; VGCC-P/Q, neuronal voltage-gated calcium channel, P/Q type.

SI conversion factors: To convert protein to grams per liter, multiply by 10; to convert white blood cells to ×10⁹/L, multiply by 0.001.

[a] Reference ranges: GAD65 antibody and VGCC-P/Q antibody, 0.02 nmol/L or less; protein, 0.35 g/L or less; white blood cells, 5/μL or less; unique oligoclonal bands, less than 4; and IgG index, less than 0.85.

[b] Bilateral optic papillitis found on examination.

Figure 2. Dual Immunostaining of Mouse Tissues With Commercial IgGs Specific for Glial Fibrillary Acidic Protein (GFAP) Intermediate Filament Isoforms and Patient IgG

A, GFAP-α and patient IgGs largely colocalize in astrocytes of pia and subpia (original magnification ×20). B, Unlike GFAP-α–specific IgG, patient IgG is largely nonreactive with radial processes of cerebellar cortical Bergmann glia (original magnification ×20). C, Both IgGs completely colocalize in myenteric plexus glia (original magnification ×20). D-F, GFAP-δ–specific IgG and patient IgG colocalize extensively in the pia and subpia (D), cerebellum (E) (note that neither GFAP-δ–specific IgG nor patient IgG binds to Bergmann glial processes), and myenteric plexus (F). Colocalized IgGs appear yellow in merged panels, and DNA is blue (4′,6-diamidino-2-phenylindole staining) (D). ε is human GFAP isoform nomenclature; δ is rodent GFAP nomenclature.

desmin being antigen. Patient IgG colocalized partially with CNS vimentin immunoreactivity, but divergent cellular staining reduced the likelihood of vimentin being antigen.

Patient IgG partially colocalized with the GFAP intermediate filament α-isoform in pial, subpial (**Figure 2**A), and subventricular astrocytes but not in GFAP-α–positive ependyma (eFigure 5 in the Supplement). Processes in myenteric plexus presumptive glia were prominently dual reactive (Figure 2C). Bergmann radial glial processes bound GFAP-α-specific IgG more intensely than patient IgG (Figure 2B). GFAP-δ/ε-specific IgG colocalized with patient IgG in all examined neu-

ral tissues: pia and subpia (Figure 2D), subventricular zones, cerebellar cortex (Figure 2E), and myenteric plexus (Figure 2F). Like patient IgG, GFAP-δ/ε-specific IgG bound to Bergmann glial filaments far less intensely than GFAP-α-specific IgG. Thus, patient IgG binding was relatively restricted to GFAP-δ/ε-expressing astrocytes.

Immunochemical Characterization of Autoantigen
Western blot probing of rat spinal cord proteins with 4 patients' IgGs revealed a common immunoreactive band (approximately 50 kDa); control human IgGs were nonreactive (**Figure 3**A).

Figure 3. Autoantigen Identification

A, Western blot of proteins isolated from rat spinal cord probed with IgG from 4 individual patients and 2 healthy controls. Patient IgG binds to an approximately 50-kDa band. B, Immunostaining of mouse periventricular region with IgG in original patient serum and with neutralized IgG acid eluted from a nonstained replica of transblotted immunoreactive band. C, Cytoplasm of glioblastoma multiforme (GBM) xenograft cells binds patient IgG, commercial glial fibrillary acidic protein (GFAP)–specific IgGs, pan-reactive, and ε-isoform–specific (green) but not control human IgG. DNA is stained blue with 4',6-diamidino-2-phenylindole. Scale bar indicates 50 μm. D, Western blot of GBM tumor xenograft lysate (8000g insoluble fraction) probed with commercial GFAP-specific IgGs and healthy control or 2 patient IgGs. E, GBM lysate proteins separated by 2-dimensional (2-D) electrophoresis and probed with IgG from 2 patients. Red outline defines the protein identified as GFAP by mass spectrometry analysis. ε is human GFAP isoform nomenclature; δ is rodent GFAP nomenclature.

Antigenicity was further demonstrated in the 50-kDa protein by reapplying to tissue sections patient IgG acid eluted from a replicate band (ie, not subjected to Western probing) (Figure 3B).

A GBM xenograft tumor cell line was identified as enriched in the human glial antigen by immunofluorescence screening of candidate glial lines with patient IgG (Figure 3C). Commercial pan-GFAP–reactive IgG, GFAP-δ/ε–specific IgG, and patient IgG, but not control human IgG, yielded filamentous cytoplasmic staining. By Western blotting, patient IgG revealed antigenicity in a GBM cytoskeletal protein (8000g insoluble fraction) (Figure 3D). Mass spectrometry analysis of common immunoreactive spots to which 2 individual patients' IgGs bound (Figure 3E) yielded partial sequences common to the N-terminal and rod domains of all GFAP isoforms. Consistent with previously reported 2-dimensional electrophoretic analysis of human brain GFAP,[10] patient IgGs bound to multiple polypeptides (interpreted to be different modification and degradation products of GFAP).

Reactivity With Isolated GFAP Isoforms

To determine whether patient IgG bound selectively to GFAP-δ/ε, GFAP-α, or isoform-common epitopes, we transfected HEK293 cells with expression plasmids encoding individual human GFAP isoforms tagged with green fluorescent protein. IgG binding analyzed on permeabilized cells (immunofluorescence) and lysates (Western blot) yielded concordant results. Commercial pan-GFAP–reactive IgG bound to both GFAP-α and GFAP-ε (Figure 4A); commercial GFAP-α–specific IgG or GFAP-δ/ε–specific IgG bound
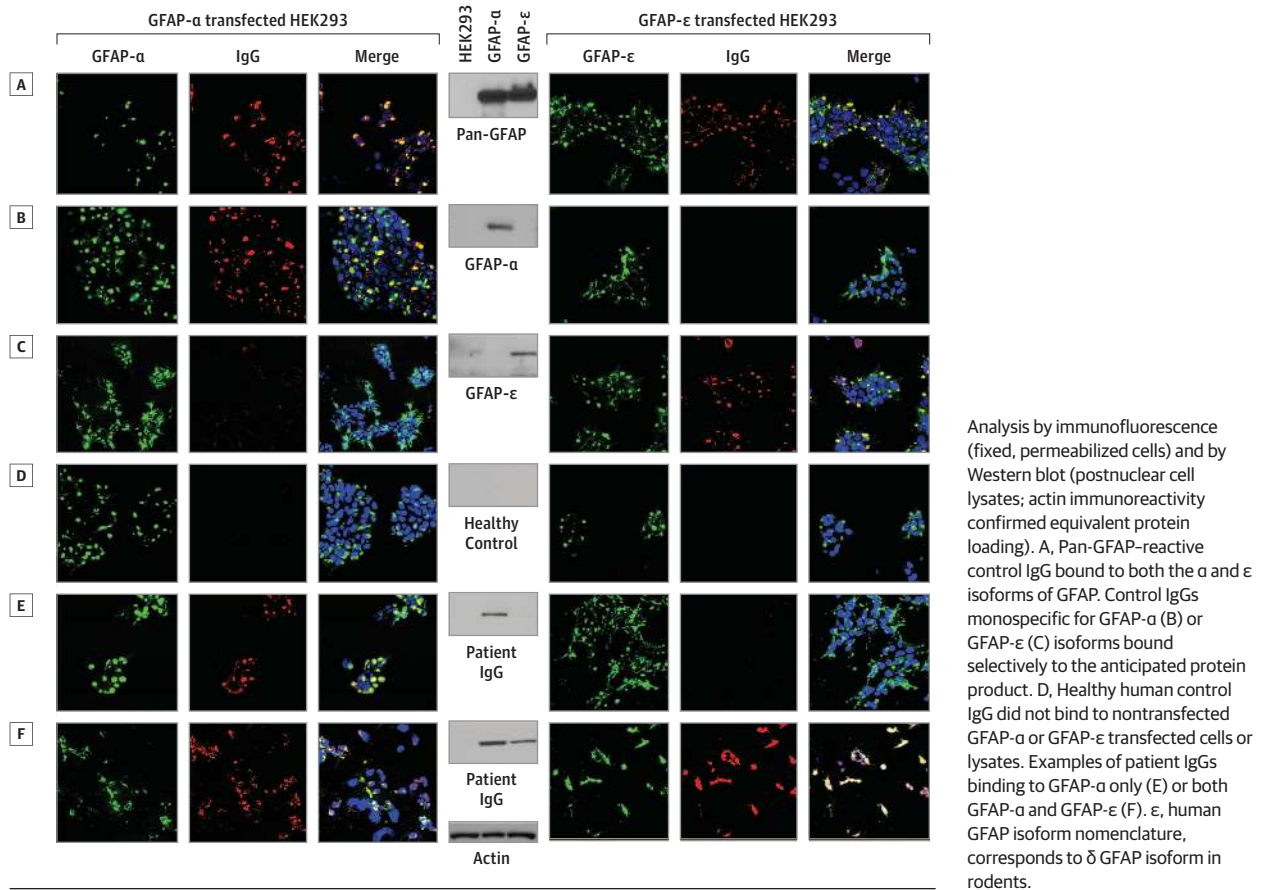
exclusively to the corresponding isoform (Figure 4B and C). IgG in only 5 of 282 control human serum specimens tested (1.8%) bound to GFAP isoform-transfected cells (Figure 4D): 3 of 135 healthy control specimens (2%), 1 of 70 specimens with miscellaneous immunopathies (1%), 1 of 57 neuromyelitis optica spectrum disorder specimens (2%), and 0 of 20 multiple sclerosis specimens. Importantly, none of those 5 bound to mouse tissue sections. Serum or CSF samples were available from 15 of 16 patients (94%) for isoform testing. IgG bound to GFAP-α cells (8 of 11 serum samples [73%] and 9 of 9 CSF samples [100%]) (Figure 4E and F). Serum IgG was dual reactive (5 of 11 specimens [45%]), solely GFAP-α reactive (3 of 11 specimens [27%]), or nonreactive (3 of 11 specimens [27%]). IgG in all 9 CSF specimens was dual reactive (Figure 4F). No serum or CSF samples were GFAP-ε monoreactive. No IgG bound to nontransfected cells. None of 49 control CSF specimens reacted with isolated GFAP.

Discussion

We identified a glial-restricted IgG autoantibody as a biomarker of an autoimmune meningoencephalomyelitis that is immunotherapy responsive. One-third of cases have serologic evidence of autoimmune endocrinopathy (some clinically evident); more than one-third are paraneoplastic. The clinical presentation is generally subacute. Headache is prominent. Common symptoms and signs are encephalitic and papillitis without increased intracranial pressure, and myelopathic. The astrocytic cytoplasmic

Figure 4. IgG Binding to HEK293 Cells Transfected With Expression Plasmids Encoding Green Fluorescent
Protein–Tagged Human Glial Fibrillary Acidic Protein α or ε Isoform

Analysis by immunofluorescence (fixed, permeabilized cells) and by Western blot (postnuclear cell lysates; actin immunoreactivity confirmed equivalent protein loading). A, Pan-GFAP–reactive control IgG bound to both the α and ε isoforms of GFAP. Control IgGs monospecific for GFAP-α (B) or GFAP-ε (C) isoforms bound selectively to the anticipated protein product. D, Healthy human control IgG did not bind to nontransfected GFAP-α or GFAP-ε transfected cells or lysates. Examples of patient IgGs binding to GFAP-α only (E) or both GFAP-α and GFAP-ε (F). ε, human GFAP isoform nomenclature, corresponds to δ GFAP isoform in rodents.

intermediate filament protein GFAP is the autoantigen. Although rarely found in healthy individuals or controls with disease, GFAP-specific IgG is a common autoantibody in neurologic practice. Tissue-based immunofluorescence is the most sensitive and specific screening assay; confirmation by a GFAP-α–transfected cell-based assay is recommended. The Mayo Clinic Neuroimmunology Laboratory's current detection rate for GFAP-specific IgG, 1 in 1000 service serologic evaluations (0.10%), equals the Purkinje cell antibody type 1 (anti-Yo) detection rate and is one-third of the detection rate for antineuronal nuclear antibody 1 (anti-Hu).[5] Glial fibrillary acidic protein–specific IgG is likely made by peripheral and CNS-infiltrating lymphoid cells. Continuing serologic observations and the frequently elevated IgG index in this initial patient cohort suggest that GFAP-specific IgG status is ascertained more sensitively in CSF than in serum. Furthermore, CSF yielded no false-positive results on GFAP-transfected cells. Accruing clinical correlative information for subsequently identified GFAP-specific IgG–seropositive patients supports the disease spectrum encountered in our 16 initially identified patients. The relatively homogeneous neurologic spectrum ascertained in blinded service screening of more than 100 000 neurologic patients predicts that GFAP-specific IgG seropositivity will distinguish autoimmune GFAP meningoencephalomyelitis from disorders commonly considered in the differential diagnosis, such as infectious,

granulomatous, and inflammatory demyelinating disorders, lymphoma, carcinomatosis, and vasculitis.

Clinical, radiologic, and CSF findings in autoimmune GFAP meningoencephalitis are reminiscent of cases reported in 1991 to 1999 as chronic or subacute corticosteroid-responsive nonvasculitic autoimmune inflammatory meningoencephalitis.[8,11,12] In those cases, CSF had lymphocytic pleocytosis and elevated IgG indexes; brain biopsies revealed perivascular lymphocytic inflammation in leptomeningeal and parenchymal vessels, without vessel wall involvement.[11,12] Veterinary investigators in Japan identified GFAP-specific IgG as a consistent CSF marker of an inflammatory canine encephalopathy.[13-17] Despite a more severe clinical course and neuropathologic findings, canine necrotizing meningoencephalitis is undoubtedly the equivalent of human autoimmune GFAP meningoencephalomyelitis. The canine presentation of severe depression and ataxia is commonly accompanied by generalized seizures, with death ensuing within months of onset.[16] Lymphocytic inflammation and cerebral cortical necrosis are prominent.[18] Magnetic resonance imaging revealed leptomeningeal enhancement in 9 of 18 canine cases.[19] The varying patterns of canine IgG reactivity with bovine GFAP fragments indicated multiple epitope specificities.[15]

As noted for classic autoimmune neurologic diseases,[1,20,21] patients with autoimmune GFAP meningoencephalomyelitis

have multiple autoimmune disorders and autoantibodies. Historically, failure to appreciate that nonneural autoantibodies and autoimmune disorders frequently coexist with neural-specific autoimmune disorders[20] has led to inappropriate naming of autoimmune neurologic disorders, for example, Hashimoto encephalopathy. Determination of the frequency and spectrum of neural autoantibodies accompanying GFAP-specific IgG awaits serologic investigation of a larger GFAP-specific IgG–positive patient cohort. It is noteworthy that serum specimens from control patients with other autoimmune disorders were mostly GFAP-specific IgG negative, except for those with autoimmune AQP4 neuromyelitis optica spectrum disorders (GFAP-specific IgG positive in 2% of cases). Association of NMDAR autoimmunity and AQP4 autoimmunity has been previously reported.[22] We detected NMDAR-specific IgG in the CSF of 2 GFAP-specific IgG–positive patients with encephalopathic features; neither had classic NMDAR encephalitis. One presentation resembled HANDL syndrome (transient headache and neurologic deficits with cerebrospinal fluid lymphocytosis). A recently described patient with NMDAR-specific IgG–positive CSF and HANDL-like clinical presentation[23] had sustained NMDAR-specific IgG seronegativity after immunotherapy and no relapse. In that report,[23] 12 archival control serum specimens from clinically diagnosed HANDL tested negative for NMDAR-specific IgG and other customarily tested neuronal IgG markers of autoimmune encephalitis (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, γ-aminobutyric acid B receptor, leucine-rich glioma inactivated 1, contactin-associated protein–like 2, and 65-kDa isoform of glutamic acid decarboxylase). Future serologic investigations should ascertain whether HANDL syndrome represents a mild clinical manifestation of autoimmune GFAP meningoencephalopathy.

It is conceivable that GFAP, AQP4, and NMDAR are coimmunogens in certain occult neoplasms and drive immune responses against all 3 antigens. The restricted binding of GFAP-specific IgG to an astroneuronal progenitor population in mouse tissue suggests the autoimmune response's initiator is a primitive neural-type cell. The 38% frequency of systemic neoplasia in GFAP-specific IgG–positive patients (continuing in prospective experience) is consistent with a paraneoplastic origin for some cases of GFAP autoimmunity. In comparison, the cancer rate for serologically negative neurologic patients evaluated in the Mayo Clinic Neuroimmunology Laboratory is 18%.[24] Glial fibrillary acidic protein immunoreactivity has been reported in some neoplasm types identified in this study: teratoma,[25] carcinoid (admixed with teratoma),[26] salivary pleomorphic adenoma,[27] prostate carcinoma,[28] and melanoma.[29] Cancer screening appropriate for age, sex, and risk factors is recommended for GFAP-specific IgG–positive patients. Detection of a coexisting antibody (eg, NMDAR-specific IgG) may refine the cancer search. Finding ovarian teratoma in 1 of 2 GFAP-specific IgG–positive cases who had NMDAR-specific IgG is consistent with the multiplicity of neural antigens in mature teratomas.[25]

Astrocyte lineage heterogeneity is well recognized.[30] With maturation, ratios of GFAP isoforms to other intermediate filament proteins change.[31,32] Human brain GFAP has 7 recognized splice variants (ε in human nomenclature is δ in rodent). In human and mouse GFAP sequences, the C-terminus of α and δ/ε isoforms diverge completely, but GFAP-α proteins are 91% identical in both species, and GFAP-δ/ε proteins are 89% identical. In the adult mouse brain, GFAP-δ is restricted to neurogenic astrocytes in subventricular areas, rostral migratory stream, and olfactory bulb,[32] locations that coincide with the distribution of patient IgG immunoreactivity.[30] Patient IgGs reactive with isolated GFAP isoforms were all GFAP-α reactive; only some bound to GFAP-ε. Immunoreactivity discrepancies observed between recombinant GFAP proteins and mouse tissue may reflect posttranslational modification of GFAP-α antigenicity in mature astrocytes or obscuring of a dominant GFAP-α epitope in tissue by 3-dimensional in situ interaction between GFAP isoforms and other intermediate filament proteins.

Conclusions

IgGs specific for intracellular autoantigens lack pathogenicity for live target cells in vivo. Their detection aids neurologic autoimmunity diagnosis[1] and provides surrogate markers of activated CD8+ cytotoxic T cells specific for peptides derived from the autoantibody-defined proteins.[33] Glial fibrillary acidic protein–derived peptides in surface major histocompatibility complex class 1 molecules upregulated on inflamed meningeal astrocytes by ambient interferon γ are plausible targets for cytotoxic T-cell attack in autoimmune GFAP meningoencephalomyelitis. Glial fibrillary acidic protein peptide–specific CD8+ T lymphocytes cause CNS inflammation in a T-cell receptor transgenic mouse model of autoimmunity.[34] Furthermore, severe meningoencephalitis developed in wild-type mice that harbored small numbers of GFAP-specific CD8+ T lymphocytes (otherwise nonpathogenic) on systemic challenge with recombinant vaccinia virus expressing the protein GFAP (otherwise nonpathogenic). Infiltrating lymphocytes localized histopathologically to meninges and vascular or perivascular space more than parenchyma. The paraneoplastic encephalomyelitis for which antineuronal nuclear antibody 2 (anti-Ri) is a biomarker is a precedent for the therapeutic benefit of corticosteroid in a CD8+ cytotoxic T-cell-mediated disorder.[35,36] An alternative explanation for impressive corticosteroid responsiveness of patients with autoimmune GFAP meningoencephalomyelitis is that GFAP-specific IgG may be accompanied by an as yet unidentified pathogenic autoantibody targeting the astrocytic plasma membrane. Pathophysiologic insights into this newly recognized autoimmune astrocytopathy are anticipated from future investigations of patient neural tissue immunopathology, T-cell antigen specificities, genetic factors, and animal models.

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