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Cerebellar Ataxia With Anti-DNER Antibodies

Outcomes and Immunologic Features

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Abstract

Background and Objectives

There is no report on the long-term outcomes of ataxia with antibodies against Delta and Notch-like epidermal growth factor–related (DNER). We aimed to describe the clinical-immunologic features and long-term outcomes of patients with anti-DNER antibodies.

Methods

Patients tested positive for anti-DNER antibodies between 2000 and 2020 were identified retrospectively. In those with available samples, immunoglobulin G (IgG) subclass analysis, longitudinal cerebellum volumetry, human leukocyte antigen isotyping, and CSF proteomic analysis were performed. Rodent brain membrane fractionation and organotypic cerebellar slices were used to study DNER cell-surface expression and human IgG binding to the Purkinje cell surface.

Results

Twenty-eight patients were included (median age, 52 years, range 19–81): 23 of 28 (82.1%) were male and 23 of 28 (82.1%) had a hematologic malignancy. Most patients (27/28, 96.4%) had cerebellar ataxia; 16 of 28 (57.1%) had noncerebellar symptoms (cognitive impairment, neuropathy, and/or seizures), and 27 of 28 (96.4%) became moderately to severely disabled. Half of the patients (50%) improved, and 32.1% (9/28) had no or slight disability at the last visit (median, 26 months; range, 3–238). Good outcome significantly associated with younger age, milder clinical presentations, and less decrease of cerebellar gray matter volumes at follow-up. No human leukocyte antigen association was identified. Inflammation-related proteins were overexpressed in the patients' CSF. In the rodent brain, DNER was enriched in plasma membrane fractions. Patients' anti-DNER antibodies were predominantly IgG1/3 and bound live Purkinje cells in vitro.

Discussion

DNER ataxia is a treatable condition in which nearly a third of patients have a favorable outcome. DNER antibodies bind to the surface of Purkinje cells and are therefore potentially pathogenic, supporting the use of B-cell–targeting treatments.

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Glossary

DNER = Delta and Notch-like epidermal growth factor–related; **HLA** = human leukocyte antigen; **IgG** = immunoglobulin G; **mRS** = modified Rankin Scale; **PCA** = paraneoplastic cerebellar ataxia.

Paraneoplastic cerebellar ataxias (PCAs) are debilitating neurologic disorders triggered by the presence of a cancer and are often associated with autoantibodies targeting intracellular neuronal antigens, such as CDR2/CDR2L (Yo antibodies), Nova-1 (Ri antibodies), or HuD (Hu antibodies).[1] Ataxia with antibodies against Delta and Notch-like epidermal growth factor–related (DNER), or DNER ataxia, is a PCA associated with Hodgkin lymphoma.[2,3] Anti-DNER antibodies correspond to a class of anti–Purkinje cell antibodies; they were known as anti-Tr antibodies until 2012, when DNER was identified as the antigenic target.[2] DNER ataxia usually presents as a severe, subacute-onset ataxia and has a male predominance.[2,3] It is important that there is no report on the long-term outcomes or whether such patients benefit from immunosuppressive treatments, although isolated cases of clinical improvement after treatment have been reported.[2,4] This is highly unusual in PCA[5-7]; a response to treatment is much more common in autoimmune limbic encephalitis, which are associated with autoantibodies that do not target intracellular proteins but cell-surface antigens and have reversible pathogenic effects.[8] It is therefore noteworthy that DNER is a single-pass transmembrane domain protein, predominantly expressed at the soma and dendrites of the cerebellum's Purkinje cells.[9] However, it is still unclear whether DNER is expressed at the cell surface of Purkinje cells, and therefore accessible to circulating antibodies, or is in instead present in organelles, within the cytoplasmic compartment.[3,10,11] The question has important therapeutic implications: if DNER is expressed at the cell surface of Purkinje cells and anti-DNER antibodies have pathogenic effects, patients may improve with antibody-depleting or B-cell–targeting treatments. To clarify these aspects, we aimed to describe the long-term outcomes of a retrospective cohort of patients with DNER ataxia, along with detailed clinical presentations, human leukocyte antigen (HLA) association, and the titers and immunoglobulin G (IgG) subclass repertoire of anti-DNER antibodies. In addition, we assessed in a cellular model whether human anti-DNER antibodies bind to DNER at the surface of live Purkinje cells.

Methods

Patients

All patients tested positive for anti-DNER antibodies in the French Reference Center for Paraneoplastic Neurological Syndromes from January 1, 2000, to December 31, 2020, were identified retrospectively. Serum and/or CSF samples were considered positive if they demonstrated a compatible staining on rat brain indirect immunohistofluorescence and stained DNER-expressing human embryonic kidney 293 cells in a cell-based binding assay, as reported elsewhere.[12] Clinical data were collected from the patients' medical records. Modified Rankin Scale (mRS) scores[13] at onset, at the time of maximum severity, and at the last visit were calculated retrospectively. The end-point dilutions of anti-DNER antibodies using a cell-based binding assay were assessed, as reported elsewhere.[12] When enough sample was available, total IgG and albumin concentrations in serum and CSF were measured using the IMMAGE 800 Protein Chemistry Analyzer (Beckman Coulter, Brea, CA), and the serum/CSF DNER antibody index was calculated using the Reiber hyperbolic formula.[14] The HLA genotypes of 20 patients were determined and compared with those of 442 healthy bone marrow donors, as reported elsewhere.[15]

Volumetric Studies

A volumetric analysis of the cerebellum was performed in all patients who had at least 2 available brain MRI scans separated by a period of at least 6 months (referred to as baseline and follow-up MRI, respectively), which included T1-weighted images. Two experienced operators (FC, SH) first performed a quality control step on all T1-weighted MRI images. A bias field correction was applied using the N4 algorithm in a 3DSlicer (slicer.org/), followed by whole-brain automatic segmentation using the SPM12 segmentation tool (fil.ionuk/spm/software/spm12/), which segments the brain into white matter, gray matter, and CSF.[16] Cerebellar segmentation was performed using the CERES cerebellum segmentation tool,[17] and cerebellar masks were combined with whole-brain white matter and gray matter masks to obtain the cerebellar white matter and gray matter masks, respectively. All masks were manually verified and manually corrected if needed at each segmentation step (WZ, SH). Volumes of the cerebellar white matter and gray matter tissues were then extracted and normalized by the intracranial volume, defined as the sum of whole-brain white and gray matter and CSF volumes. The total cerebellar volumes were calculated by adding normalized cerebellar white and gray matter volumes.

Organotypic Cerebellar Slices

Postnatal day 9 Wistar rats (RjHAn-Wistar; Janvier Labs, Le Genest Saint Isle, France) were deeply anesthetized with isoflurane and decapitated. Vermes were dissected in cold phosphate-buffered saline enriched with calcium/magnesium and 6 mg/mL D-glucose, and cut into 350-μm thick sagittal slices using a McIlwain tissue chopper (World Precision Instruments, Sarasota, FL). Slices were placed in Millicell organotypic tissue culture inserts (Millipore-Sigma, Burlington, MA) and kept at 37°C in serum-containing medium (50% minimal essential medium with Earle's, 25% heat-inactivated horse serum, 25% Hank's BSS supplemented with L-glutamine, penicillin-streptomycin, 6 mg/mL D-glucose), which was gradually switched to serum-free medium (50% DMEM without phenol red, 50% Ham's F12 supplemented with 1% B27 and 0.5% N2, L-glutamine, penicillin-streptomycin, 6 mg/mL D-glucose) over

the first week of culture. For immunostaining, slices between day in vitro 33 and day in vitro 50 were incubated overnight at 37°C with CSF (1:50) from an anti-DNER antibody patient or from a control subject. After washing, slices were fixed with 4% paraformaldehyde, permeabilized, and incubated with a mouse anti-calbindin D-28K antibody (1:1,000, 214,011, Synaptic system, Göttingen, Germany). Autoantibody reactivity was revealed using appropriate secondary antibodies (Alexa-Fluor 488 goat anti-human IgG, A11013; Invitrogen, Courtaboeuf, France, 1:1,000 and Alexa-Fluor 555 goat anti-mouse IgG, 10143952; Fisher Scientific, Illkirch, France, 1:1,000). Alternatively, slices were fixed and permeabilized first, followed by coincubation with a patient's CSF (1:20), a rabbit antibody targeting the DNER C-terminus (ab191913; Abcam, Amsterdam, Netherlands, 1:400) and a mouse anti-calbindin D-28K antibody (1:1,000), overnight at 4°C, followed by secondary antibodies. Rabbit anti-DNER antibody binding was revealed using a goat biotinylated anti-rabbit IgG (1:400, BA-1000, Eurobio, Courtaboeuf, France), followed by Alexa Fluor 647-conjugated streptavidin (1:200, 532,357, Invitrogen). Slices were mounted on slides and scanned using a confocal microscope (LSM 880, Zeiss, Marly le Roi, France).

Cell Membrane Isolation and Western Blotting

A 7-week-old male Oncins France Strain A Sprague Dawley rat (Charles River Laboratories, Saint-Germain-sur-l'Arbresle, France) was deeply anesthetized with isoflurane and decapitated. The brain was collected, quickly frozen in liquid nitrogen, ground in a ceramic mortar, and suspended in lysis buffer (0.32 M sucrose, 2 mM ethylenediaminetetraacetic acid, 20 mM Tris; and protease inhibitor cocktail, 04693132001, Sigma-Aldrich, Saint-Quentin-Fallavier) at 4°C. After sonication, the homogenate was centrifugated (1,000 g, 10 minutes; 12,000 g, 20 minutes; 100,000 g, 1 hour; 4°C) to separate cytosolic proteins from the membrane fraction. For western blotting, 10 μg of proteins were loaded on 4%–12% precast gel (3450124; Bio-Rad, Marne La Coquette, France). After migration, proteins were transferred on a nitrocellulose membrane subsequently incubated with rabbit anti-DNER antibody (1:1,000), rabbit anti-calpain antibody (1:1,000, C5986; Sigma-Aldrich), or mouse anti-pancadherin antibody (1:1,000, C1821; Sigma-Aldrich) and revealed with appropriate secondary antibodies (horseradish peroxidase–coupled goat anti-rabbit IgG, 1:1,000, 111-036-003, or horseradish peroxidase–coupled goat anti-mouse IgG, 1:1,000, 115-036-003; Jackson ImmunoResearch, Ely, United Kingdom).

Proteomic Analysis

The relative expression levels of 643 proteins were assayed in the CSF of healthy controls (n = 40) and DNER ataxia patients (n = 5) using a synthetic single-stranded DNA-based molecular recognition (SOMAmer) elements-based approach, as reported elsewhere.[15] As the measurement of the variance depends on the relative abundance of each protein, raw expression values were log2-transformed to reduce heteroskedasticity. Comparison with CSF samples from other types of PCAs was not possible because of limited sample availability.

Statistical and Bioinformatics Analysis

All statistical analyses were performed using R version 4.1.2 and RStudio v1.4 1717. Continuous variables are expressed as median and range, and discrete variables as number and percentage. Comparison of medians was performed using the unpaired Wilcoxon test, except for volumetric analyses for which the paired Wilcoxon test was used; the results were not adjusted for multiple comparisons considering the exploratory nature of this study. Differences in HLA carrier frequencies between patients and controls were analyzed by a 2-tailed Fisher exact test. The Bonferroni method was used to correct for multiple comparisons according to the number of alleles of each locus. p-values ≤0.05 were considered significant. Univariate analysis of proteomic data was performed using the Wilcoxon test for unpaired comparisons, and the Benjamini-Hochberg procedure for multiple comparison correction. Only proteins with an adjusted p-value ≤ 0.01 were considered as differentially expressed. Supervised hierarchical clustering was performed using Euclidean distances and the Ward linkage method on the Z-score of the $\log_2$-transformed measurement.

Standard Protocol Approvals, Registrations, and Patient Consents

This study is part of the project Gene PNS (*NCT03963700*) and was approved by the institutional review board of the Hospices Civils de Lyon. Biological samples were collected after obtention of patient's written consent.

Data Availability

Data supporting our findings will be available on request.

Results

Clinical Presentations

Twenty-nine patients with DNER antibodies were identified between 2000 and 2020. Data could not be retrieved for 1 patient; 28 patients were therefore included in this study. The median age at disease onset was 52 years (range 19–81) and 23 patients (82.1%) were male (sex ratio, 1:4.6). Progression of disease was subacute in most of the patients (26/28, 92.8%), and most of them became moderately to severely disabled (mRS at maximal severity >2 in 27/28, 96.4%). All-but-one patient presented with cerebellar ataxia, the remaining one developed acute confusion that receded after the identification and treatment of Hodgkin lymphoma. In addition, 16 of the 28 patients (57.1%) had noncerebellar features, including cognitive impairment (confusion, n = 2; psychomotor slowing, decreased verbal fluency, and/or impaired mental flexibility, n = 4) and seizures (n = 2, one patient with 2 generalized tonic-clonic seizures 7 months before ataxia onset; another who developed temporal lobe epilepsy 31 months after disease onset). Moreover, 11 of the 28 patients (39.3%) had peripheral neurologic symptoms, including pain, paresthesia, hypoesthesia, sensory ataxia, and/or fasciculations and amyotrophy; these symptoms

Table 1 Clinical Features Patients With Anti-DNER Antibody, Stratified by Outcome Categories

	All patients, n = 28	Patients with favorable outcome (mRS at the last visit ≤2), n = 9	Patients with poor outcome (mRS at the last visit >2), n = 19	*p* Values
Median age at onset, y (range)	52 (19–81)	44 (24–60)	56 (19–81)	0.025
Male sex, n (%)	23 (82.1)	8 (88.9)	15 (78.9)	0.910
Progression of disease, n (%)				
Subacute (1d–3 mo)	26 (92.8)	9 (100)	17 (89.4)	1.000
Chronic (≥3 mo)	2 (7.1)	0	2 (10.5)	1.000
Cerebellar symptoms, n (%)				
Gait ataxia	27 (96.4)	8 (88.9)	19 (100)	0.697
Limb ataxia	21 (75)	3 (33.3)	18 (94.7)	0.002
Nystagmus and/or oscillopsia	19 (67.9)	6 (66.7)	13 (68.4)	1.000
Dysarthria	19 (67.9)	2 (22.2)	17 (89.5)	0.002
Cognitive impairment	6 (21.4)	1 (11.1)	5 (26.3)	0.673
Seizures, n (%)	2 (7.1)	0	2 (10.5)	0.908
Peripheral neurologic involvement, n (%)	11 (39.3)	3 (33.3)	8 (42.1)	0.976
Cerebrospinal fluid, n/N (%)				
Normal	5/25 (20)	1/7 (14.3)	4/18 (22.2)	1.00
Increased WBC >5/μL	17/25 (68)	5/7 (71.4)	12/18 (66.7)	1.00
Increased protein level ≥60 mg/dL	9/25 (36)	1/7 (16.7)	7/18 (53.8)	0.305
Positive oligoclonal bands	4/16 (25)	1/6 (16.7)	4/10 (40)	0.676
Tumor association, n (%)				
Hodgkin lymphoma	20 (71.4)	8 (88.9)	12 (85.7)	1.00
Other malignancy[a]	4 (14.3)	1 (11.1)	3 (20)	1.00
No tumor, n (%)	4 (14.3)	0	4 (21.1)	0.364
Median follow-up, mo (range)	26 (3–238)	46 (14–201)	21 (3–238)	0.09
Median mRS score (range)				
At onset	3 (1–5)	1 (1–5)	3 (1–4)	0.202
At maximum severity	4 (2–5)	3 (2–5)	4 (3–5)	0.012
At the last visit	4 (0–6)	1 (0–2)	4.5 (3–6)	—

Abbreviations: mRS = modified Rankin Scale; WBC = white blood cell.
[a] Anaplastic large T-cell lymphoma, 2 patients; chronic lymphocytic lymphoma, 1 patient; unproven pancreatic tumor, 1 patient.

appeared before the onset of chemotherapy in 5 of them (Table 1). Electromyographic investigations found a length-dependent sensory axonal and/or demyelinating polyneuropathy in 5 of the 8 patients (62.5%). CSF analysis showed increased white blood cell CSF counts and/or oligoclonal bands in most of the analyzed patients (18/25, 72.0%; Table 1). Cerebellar atrophy on brain MRI was reported in the medical chart of 7 of the 27 patients (25.9%); one additional patient had a T2-weighted hyperintense signal of the cerebellar parenchyma without gadolinium enhancement.

Association With Hodgkin Lymphoma and Other Hematologic Malignancies

All patients had whole-body 14-fluorodeoxyglucose PET, identifying a biopsy target in 24 patients (85.7%, lymph node, n = 22; thymus, n = 1; pancreas, n = 1). A hematologic malignancy was found in 23 patients (82.1%, biopsy performed twice in 3 patients due to small target size): Hodgkin lymphoma (20/28, 71.4%; 13 were the classic nodular-sclerotic subtype), $CD30^+$ anaplastic large T-cell lymphoma (2/28, 7.1%), and chronic lymphocytic leukemia (1/28, 3.6%). In only 1 patient, Hodgkin lymphoma was known before the apparition of neurologic

Table 2 Immune-Modulating Treatments

	All patients, n = 28	Patients with favorable outcome (mRS at the last visit ≤2), n = 9	Patients with poor outcome (mRS at the last visit >2), n = 19	*p* Values
Median time to first treatment, d (range)	41 (5–2,387)	41 (16–67)	42 (5–2,387)	0.688
Corticosteroids, n (%)	7 (25)	2 (22.2)	5 (26.3)	1.000
Intravenous immunoglobulins, n (%)	20 (71.4)	6 (66.7)	14 (73.7)	1.000
Rituximab, n (%)	11 (39.3)	6 (66.7)	5 (26.3)	0.104
Intravenous cyclophosphamide, n (%)	4 (14.3)	0	4 (21.1)	0.364
Plasmaphereses, n (%)	2 (7.1)	0	2 (10.5)	0.822
No treatment, n (%)	5 (17.9)	2 (22.2)	3 (15.8)	1.000

Abbreviation: mRS = modified Rankin Scale.

symptoms, which appeared at the time of an oncologic relapse. One other patient (4.3%) had a suspicion of solid neoplasm (pancreas), not histologically confirmed (Table 1). Most lymphoma patients had local or locally extended disease (Ann Arbor stage I–II; 18/22, 81.8%). All patients with histologically proven neoplasms (23/28, 82.1%) underwent chemotherapy, usually combining doxorubicin, bleomycin, vinblastine, and dacarbazine (17 patients). Ten patients (43.5%) received targeted therapies (rituximab, 5/10; brentuximab, 2/10; pembrolizumab, 1/10). Treatment with pembrolizumab in 1 patient did not result in neurologic worsening. Twenty patients (86.9%) achieved complete response. Two patients had a neoplastic relapse during follow-up, without neurologic relapse. Of the 4 patients with no identified cancer (14.3%), 2 were followed up for more than 2 years, 1 died 3 months after diagnosis, and 1 was lost to follow-up 3 months after disease onset.

Treatments and Long-term Outcomes

The median duration of follow-up was 26 months (range 3–238, Table 1). Twenty-three patients (82.1%) received immune-modulating treatments; the most frequent was IV immunoglobulins (20/28, 71.4%) followed by rituximab (11/28, 39.3%; Table 2). Five (18%) patients died of nonneurologic causes, and 2 patients (7.1%) experienced a neurologic relapse, 13 and 20 months after disease onset (both previously treated with IV immunoglobulins and rituximab). Fourteen patients (50.0%) improved after treatment, with a reduction of 1–4 points of mRS from maximum disability to last visit (median, 1 point). Patients began to improve a median 2.7 months after onset (range, 11 days to 18 months). At the last visit, 9 of the 28 patients (32.1%) had a favorable outcome (no or slight residual disability; mRS ≤2); 7 of these favorable outcome patients had severe disability (mRS >2) at baseline. None of the patients had extracerebellar features at the last visit, whereas 27 of the 28 patients (96.4%) had persisting symptoms of truncal and/or appendicular ataxia. As compared with patients with poor outcomes, those with favorable outcome were significantly younger (p = 0.025), had a lower median mRS score at maximum disease severity (p = 0.012), and had less extensive cerebellar involvement (i.e., less frequent limb ataxia and less frequent dysarthria, both p = 0.002). In addition, there was a trend toward more frequent use of rituximab in those with favorable outcome than in those with poor outcome (6/9, 66.7% vs 5/19, 26.3%; Table 2) and toward a longer median follow-up (46 vs 21 months; Table 1).

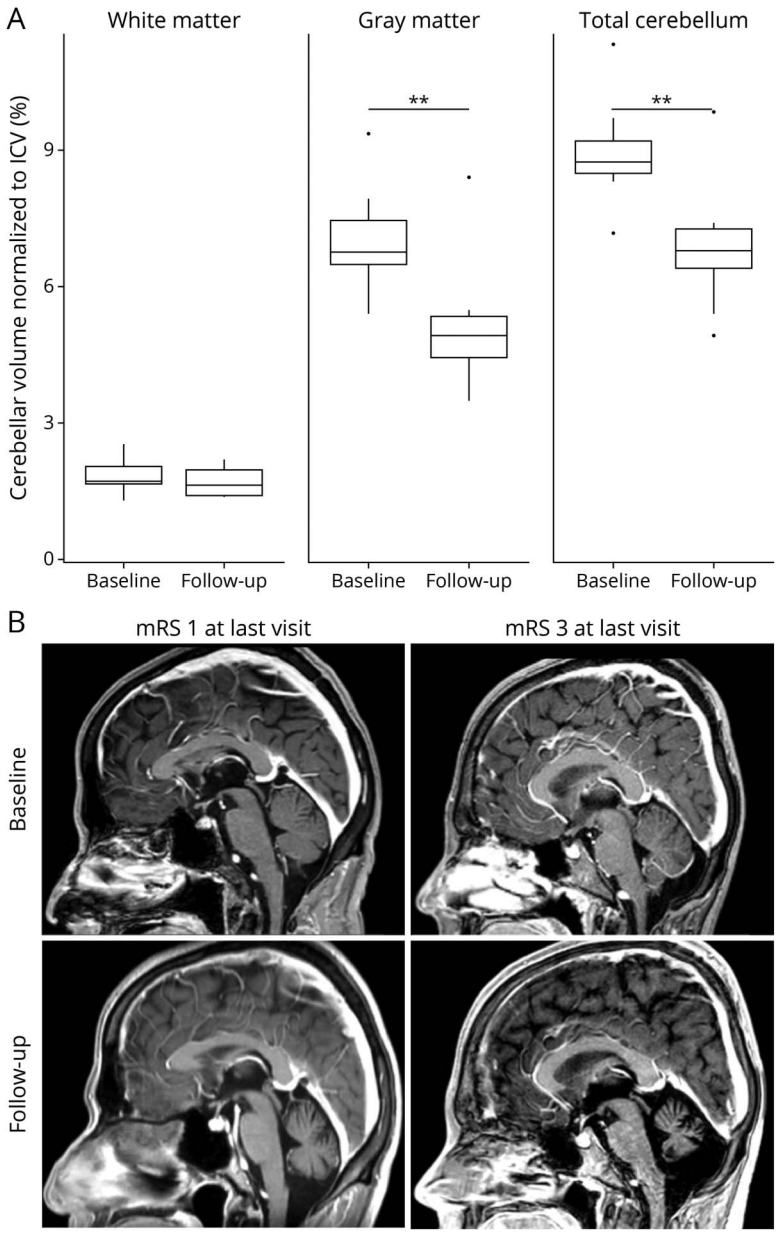
Longitudinal Volumetry of the Cerebellum

Longitudinal brain MRIs including 3D T1-weighted images were available for 10 patients (35.7%), including 5 with favorable outcome (mRS ≤2 at the last visit). The duration of follow-up and time to MRIs were similar in patients with poor and favorable outcomes (eTable 1, links.lww.com/NXI/A737). All images were gadolinium-enhanced. Compared with baseline, there was a significant decrease in the median total cerebellar volumes and cerebellar gray matter volumes at follow-up (Figure 1A). Moreover, the decrease of cerebellar gray matter volumes at follow-up was more important in patients with poor outcome (mRS >2 at the last visit) compared with patients with favorable outcome (mRS ≤2 at the last visit; p = 0.016; Figure 1, B and C, eTable 1, links.lww.com/NXI/A737). Taking into account the interval between baseline and follow-up MRI, the median decrease of cerebellar gray matter was also significantly faster in patients with poor outcome (p = 0.016; eTable 1, links.lww.com/NXI/A737).

Immunologic Studies

Anti-DNER antibodies were detected in the serum of 22 of the 25 patients (88%) and in the CSF of 24 of the 24 patients (100%). Three patients (12%) had DNER antibodies only in CSF. Twenty sera (71.4%) and 9 CSF (32.1%) were available for titration and subclass repertoire analysis. The median end-point dilutions of DNER antibodies were 1:3,200 (range, 1:100–1:40,9600) in serum and 1:200 (range, 1:40–1:12,800) in CSF. Intrathecal synthesis of anti-DNER antibodies (antibody index >1.4) was found in 7 of the 7 analyzed patients. DNER-specific IgG antibodies were predominantly of the IgG1 and IgG3 subclasses in both serum and CSF (eFigure 1, links.lww.com/NXI/A737). Regarding HLA, there was no significant difference in allele carrier frequencies between patients and controls for either HLA Class I (A, B, C) or Class II (DRB1, DQB1, DQA1, and DPB1) genes (not shown). Differential protein expression analysis found 13

Figure 1 Longitudinal Volumetric Analysis of the Patients' Cerebellum



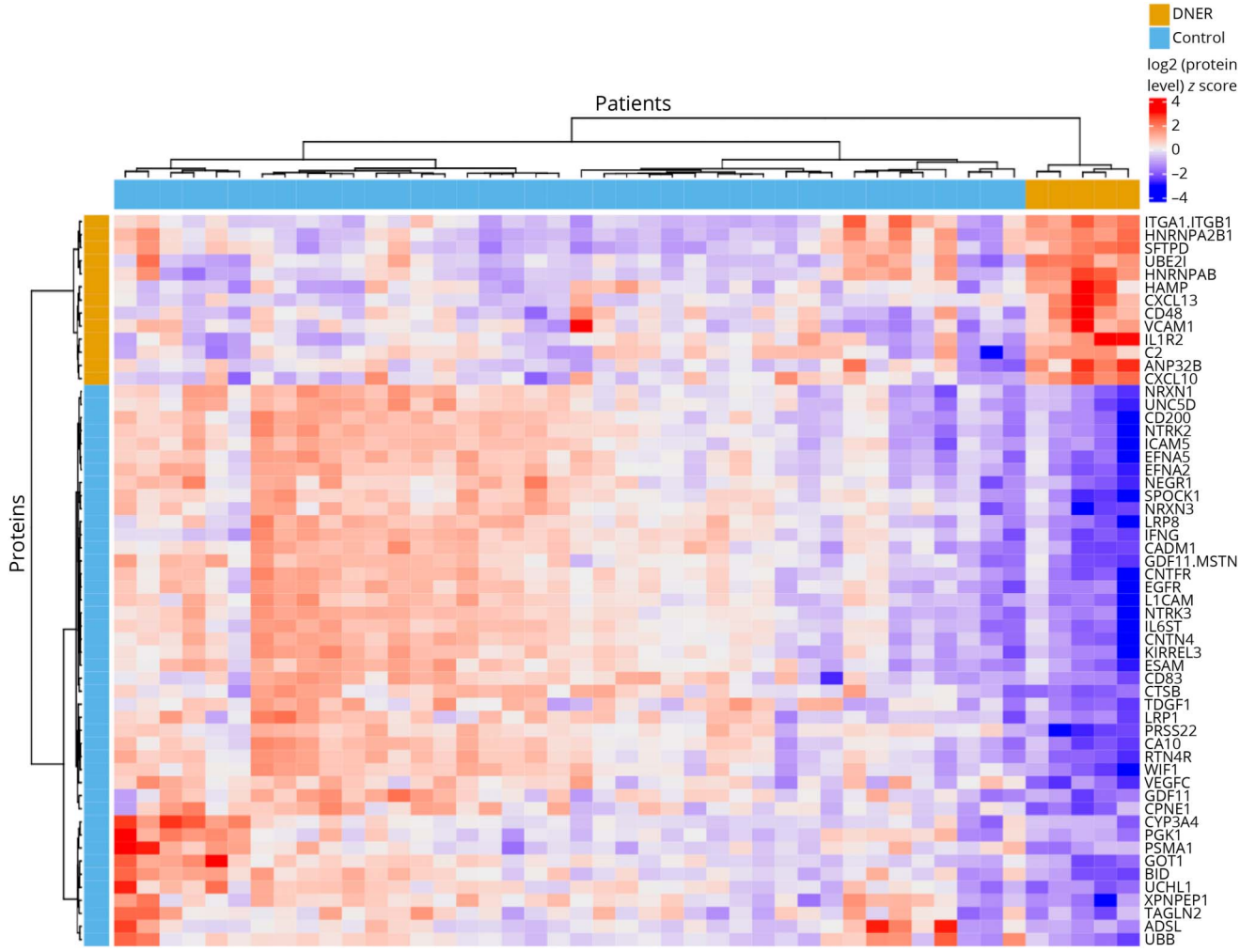
(A) Boxplot showing the volumes of cerebellar white matter, cerebellar gray matter, and whole cerebellum at baseline and follow-up, in 10 patients with anti-DNER antibodies. Volumes are expressed as percentages of intracranial volume. There is a significant decrease of cerebellar gray matter and whole-cerebellum volumes at follow-up, compared with baseline, $^{**}p < 0.005$. (B) Representative images of sagittal T1-weighted brain MRI at baseline and follow-up of a patient with favorable outcome (follow-up MRI 9 months after disease onset) and a patient with poor outcome (follow-up MRI 6 months after disease onset).

overexpressed and 43 underexpressed proteins in the CSF of 5 DNER ataxia patients compared with controls. Overexpressed proteins were mainly related to B-cell immune response (i.e., C-X-C motif chemokine 13 or CXCL13, CD48), and inflammation (including hepcidin, complement component 2, and the neuron-specific inflammatory chemokine CXCL10), or reflected neuronal tissue lysis (i.e., heterogeneous nuclear ribonucleoproteins A2/B1, SUMO-conjugating enzyme UBC9, and acidic leucine-rich nuclear phosphoprotein 32 family member B). Conversely, interferon gamma was downregulated compared with controls; other underexpressed proteins were mostly related to neurodevelopment and cell cycle (Figure 2).

DNER Antibodies Bind Live Purkinje Cells In Vitro

Incubation of live organotypic cerebellar slices with an anti-DNER antibody–positive CSF showed the binding of human IgG at the surface of the soma and dendrites of live Purkinje cells (Figure 3A). In fixed and permeabilized organotypic cerebellar slices, similar immunolabeling with a patient CSF was superposable to the staining obtained with a commercial antibody targeting the C-terminus of DNER (Figure 3B). Furthermore, fractionation of rat brain lysates found an enrichment of DNER in the membrane fraction only, which was not detected in the cytosol fraction (eFigure 2, links.lww.com/NXI/A737).

Figure 2 Proteomic Analysis of the Cerebrospinal Fluid of Patients With DNER Ataxia

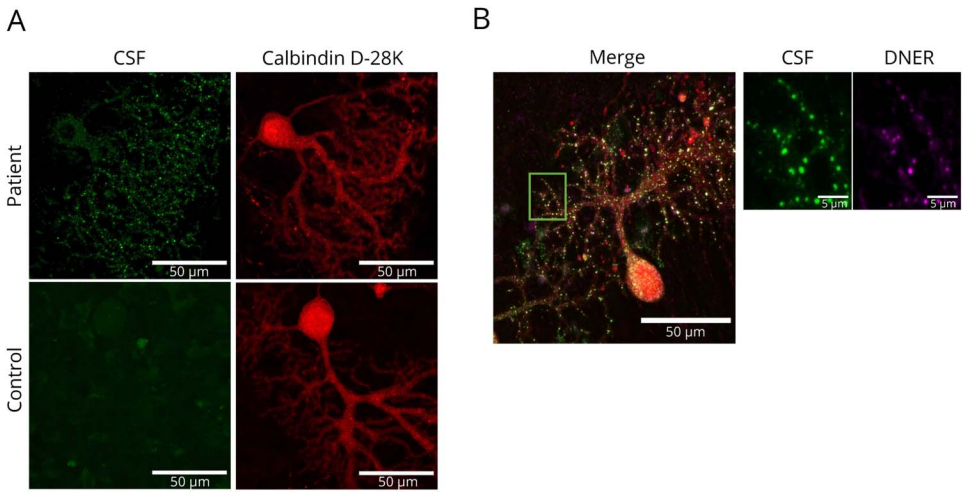


Heatmap of CSF $\log_2$ expression of proteins shown as median Z-scores per protein in patients with anti-DNER antibodies (n = 5) compared with controls (n = 40). Overexpressed proteins were mainly related to immune response. Among them, overexpression of C-X-C motif chemokine 13 (CXCL-13) and CD48 is implicated in B-cell activation. Other immune-related proteins included complement component 2 (C2), interleukin-1 receptor type 2 (IL1R2), chemokine C-X-C motif chemokine 10 (CXCL10), pulmonary surfactant-associated protein D (SFtPD), and hepcidin (HAMP). Proteins of leukocyte migration were also overexpressed, including vascular cell adhesion protein 1 (VCAM-1), and integrin alpha1 and beta1 (ITGB1/ITGA1). Other overexpressed proteins likely reflected tissue lysis, including proteins involved in cell cycle or processing of proteins and RNA, such as heterogeneous nuclear ribonucleoproteins A2/B1 (HNRNPA2B1), SUMO-conjugating enzyme UBC9 (UBE2I), and acidic leucine-rich nuclear phosphoprotein 32 family member B (ANP32B). Underexpressed proteins were interferon gamma (IFNG), and proteins mostly related to neural development (such as neuronal growth regulator 1 or NEGR1 and the neurexins 1 and 3: NRXN1, NRXN3) or cell cycle (e.g., BH3-interacting domain death agonist [BID]).

Discussion

This study demonstrates that DNER ataxia is a treatable disorder: half of the patients included herein improved after treatment, and at the last visit, nearly a third of them had recovered functional independence. This is of importance considering the long survival of patients with limited Hodgkin lymphoma.[18] It is also in contrast to other paraneoplastic ataxias: most of the patients with Yo, Ri, and Hu ataxia not only are bedridden during the plateau phase but also improve very rarely, if at all, at the long term.[5,7,19,20] Furthermore, those with favorable outcome had less extensive cerebellar involvement and clinical severity at onset and were younger. Because of the small sample size, this study is exploratory and these results were not adjusted for multiple testing; larger studies are needed to confirm them. In addition, we found a relationship between poor outcome and the decrease of cerebellar gray matter volumes, suggesting that the long-term persistence of ataxic symptoms in patients is due to irreversible neuronal loss. Identifying the causes of such neuronal damage is necessary to improve treatment strategies, and importantly, several findings in this study suggest a direct role of anti-DNER antibodies. One important point is the cell-surface localization of DNER and its accessibility to circulating autoantibodies. Although anti–Purkinje cell antibodies were identified as early as 1976 in patients with Hodgkin lymphoma-associated ataxia (under the name of anti-Tr antibodies), they were long considered devoid of any pathogenic effect because they were believed to target an intracellular protein.[11,21,22] When DNER was finally identified as the target antigen, a cell-surface localization was considered because of the structure of the protein but was not clearly

Figure 3 DNER Antibodies Bind DNER at the Surface of Purkinje Cells



(A) Incubation of nonpermeabilized live organotypic cerebellar cultures with an anti–DNER-positive CSF shows punctuate cell-surface immunolabeling (top) that is not obtained with a control CSF (bottom). Purkinje cells were labeled with an anti-calbindin D-28K antibody (red). (B) Fixed and permeabilized organotypic cerebellar cultures labeled with an anti-DNER-positive CSF (green), a commercial anti-DNER antibody (purple), and calbindin D-28K (red). Commercial anti-DNER and human DNER-specific IgG demonstrate superposable staining of the dendrites and soma of Purkinje cells.

demonstrated.[2,9,10] The findings herein show not only that DNER has a predominant cell-surface expression but also that anti-DNER antibodies from the patients, which target conformational epitopes,[2] bind the surface of live Purkinje cells. In addition, anti-DNER antibodies were detected in all the CSF samples included herein, and intrathecal synthesis of DNER-specific IgG was demonstrated in all analyzed patients, suggesting that anti-DNER antibodies are present near the cerebellar parenchyma. Moreover, proteomic analysis of the patients revealed upregulation of B-cell–related proteins, hinting at a prominent role of antibody-producing cells, and downregulation of interferon gamma, a pivotal cytokine in T-cell activation.[23] These findings are similar to previous reports on other neuroinflammatory diseases, such as anti-NMDAR encephalitis, MS, and neuromyelitis optica spectrum disorder,[24-26] and may explain the trend observed herein toward more favorable outcome in rituximab-treated patients. It may also be because rituximab was used in more than a third of the patients that most of them improved, compared with only 4 of the 28 patients (14.3%) in a study published 2 decades ago.[4] It is important that anti-DNER antibodies from the patients included herein were predominantly of the proinflammatory IgG1 and IgG3 subclasses.[4,27,28] IgG1 and IgG3 subclasses bind efficiently most Fcγ receptors, and IgG3 are particularly strong activators of complement.[29] Purkinje cell loss was reported in autopsic reports of patients with antibodies against DNER or other Purkinje cell-surface proteins, such as voltage-gated calcium channels.[30] Although brain deposition of complement was not investigated in these studies, the present findings suggest that cell-surface autoantibodies bound to Purkinje cells can mediate antibody-dependent and/or complement-dependent cytotoxicity against Purkinje cells. Alternative pathogenic effects to consider include antibody-mediated internalization of cell-surface DNER, and/or perturbations of the (yet unknown) signaling function of DNER, similarly to the effects of autoantibodies in anti-mGluR1 ataxia[31,32]; however, they are less likely to explain the development of cerebellar atrophy. Further studies are needed to confirm the pathogenic effects of anti-DNER antibodies and characterize them.

Regarding the clinical presentations, while the majority of the patients in the present cohort had subacute-onset ataxia, more than half of them also presented with noncerebellar features, such as seizures or cognitive impairment. Limbic encephalitis was reported in a patient with anti-Tr antibodies, and it is possible that a rare minority of patients with anti-DNER antibodies develop limbic encephalitis.[4] However, most of the cognitive symptoms from the present cohort were compatible with cerebellar cognitive-affective syndrome[33]; the cognitive aspects of cerebellar dysfunction were acknowledged relatively recently,[33,34] and further studies are needed to estimate their importance in immune-mediated ataxias. As for seizures, these were observed in only 2 patients and did not develop at the same time as ataxia; a causal link with anti-DNER autoimmunity is only speculative. The peripheral neurologic symptoms observed in some patients were likely coincidental and were possibly aggravated by neurotoxic agents such as vincristine; however, their apparition before the onset of chemotherapy in some patients is intriguing.

It is important that we did not identify an association with HLA that could be involved in the development of anti-DNER autoimmunity, in line with previous studies on paraneoplastic neurologic syndromes, suggesting that tumor-related specificities are likely to be more relevant.[35,36] As previously reported, the most common tumor association was limited Hodgkin lymphoma,[2,4] but with demographics unusual for this disease:

the age distribution was in-between the 2 incidence peaks of the classically bimodal distribution of this hemopathy,[37] and there was an overwhelmingly male predominance, when in classical Hodgkin lymphoma, sex distribution is more balanced.[18] These atypical features suggest that in these patients, the tumors harbor distinctive molecular alterations that may participate in the breakdown of immune tolerance. Conversely, overexpression of DNER in the tumors is probably not involved because DNER antibody immunoreactivity was observed in only 2 of the 16 tumor samples investigated in the literature.[4,38]

To conclude, this study found that DNER ataxia is a treatable paraneoplastic neurologic disorder. Moreover, the ability of anti-DNER antibodies to bind the surface of Purkinje cells, and their proinflammatory features, suggests that they are likely pathogenic, supporting the use of B-cell–targeting treatments in the affected patients. Further studies are warranted to confirm and characterize the pathogenicity of anti-DNER antibodies and to understand the role of tumors in the development of anti-DNER autoimmunity.

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Disclosure

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Continued

Appendix *(continued)*

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Appendix *(continued)*

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