Short Communication

Microglial and Neuronal TDP-43 Pathology in Anti-IgLON5-Related Tauopathy

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Abstract. A novel neuronal tauopathy, mainly confined to hypothalamus and brainstem tegmentum, has recently been reported in patients with autoantibodies to the neuronal cell-adhesion molecule IgLON5. We describe a patient with anti-IgLON5 syndrome, who presented with dysautonomia and sleep disorder, followed by subacute dementia. Postmortem brain examination disclosed neuronal tau pathology prevailing in the hippocampus, amygdala, and locus coeruleus, in addition to microglial/neuronal TDP-43 pathology, with overexpression of aberrantly phosphorylated forms and neurotoxic truncated fragments, in basal ganglia, nucleus basalis, thalamus, and midbrain. These findings suggest that neurodegeneration in anti-IgLON5 syndrome might also occur via a microglia-triggered non-cell autonomous pathway.

Keywords: IgLON5, microglia, non-cell autonomous neurodegeneration, tauopathy, TDP-43 pathology

INTRODUCTION

Autoantibodies against IgLON5, a cell adhesion molecule belonging to the Ig superfamily, have recently been discovered in eight patients with a distinctive neurodegenerative disorder combining parasomnia, motor disturbances, dysautonomia, and brainstem signs [1]. Disease progression occurred over 2–12 years in the majority of cases, with the exception of two patients who presented a rapidly progressive course and died within 6 months from disease onset. A striking neuropathological feature of this condition, as assessed in two cases with disease duration of 6 years and 6 months, respectively, was the detection of widespread tau pathology under pretangles, tangles, and neuropil threads, predominantly in hypothalamic nuclei and brainstem tegmentum; in the subject with shorter disease duration, severe tau pathology was also detected in the cervical spinal cord, in addition to isolated Bunina bodies and neuronal TDP-43 aggregates in the brainstem and anterior horn neurons of the spinal cord. Here we report on neuropathological and biochemical changes detected in “patient 4” of the above cohort [1].

PATIENT AND METHODS

Patient

A 69-year-old right-handed woman was admitted to the Neurology Unit, University Hospital of Padova,
for recurrent episodes of syncope. Over the previous three months, anxiety, obsessive thinking, compulsions, and bouts of daytime sleep, were reported; in addition, night-time sleep was fragmented and characterized by repetitive lip sucking, continuous limb movements, sleep-talking, and transient intense snoring. Her medical history was notable for uncomplicated mild hypertension and bilateral low-grade carotid stenosis, for which she was in treatment with enalapril and aspirin. No evidence of cognitive decline was reported by family members during the months preceding the onset of neuropsychiatric symptoms and sleep disturbances. On admission, the patient was alert and collaborative but not oriented to place or time. Akathisia and subcontinuous dyskinetic movements of the limbs were observed. Deep-tendon reflexes were brisk and symmetrical and motor strength was 5 out 5 throughout. There were neither sensory deficits nor cerebellar impairment. Mild vertical supranuclear ophthalmoplegia with intact vestibulo-ocular reflexes and hoarseness were noted. The patient scored 21 on Mini-Mental State Examination (MMSE). During her stay in the Neurology Unit, the patient has been falling asleep repeatedly while eating, reading, and during morning round. Routine blood tests, results of thyroid function, and an extensive workup for infectious, metabolic, inflammatory, paraneoplastic, and dysimmune etiologies were unrevealing (results of antibodies to IgLON5 were known after death). A lumbar puncture revealed clear cerebrospinal fluid (CSF) with 5 white cells per microliter and normal protein and glucose levels; a 14-3-3 protein assay was negative, total tau was 236 pg/ml, and no oligoclonal bands were detected. Sequencing of the prion protein gene revealed no mutations and methionine homozygosity at polymorphic codon 129. During an electroencephalographic (EEG) recording, the patient fell asleep several times and on these occasions only transient fragmentation of the alpha rhythm was observed. Overnight polysomnography revealed scarce K complexes and rare and short desynchronization and slowing patterns, against a continuous 9-10 Hz background rhythm. Throughout the night, she presented flexion-extension movements, greater on the left limbs. Brain MRI was normal. Total body $^{18}$F-fluorodeoxyglucose positron-emission tomography ($^{18}$FDG PET) revealed only gastric tracer accumulation, a finding consistent with chronic gastritis detected at esophagogastroduodenoscopy; no brain $^{18}$FDG PET scanning was performed. An electrocardiogram (ECG) was normal and blood-pressure monitoring revealed mild hypertension with an inverted sleep-wake pattern. Six months later, the patient was brought to the emergency room because of unconscious state, hypercapnic acidosis, and hypoxia. After appropriate treatment, the patient was admitted to the Neuropsychological assessment revealed a score of 16 on MMSE; in addition, visual agnosia, hallucinations, and delusions were observed. No other changes in neurological examination were recorded. Routine blood tests were normal, whereas a marked increase in noradrenaline (681 pg/mL, n.v. 185 ± 40), morning cortisol (1219 nmol/L, n.v. 138–690), and ACTH (90 ng/L, n.v. 10–50) levels were consistent with a hypersympathetic tone. Repeated CSF analysis and brain MRI were unremarkable. Continuous ECG monitoring revealed a normal sinus rhythm interspersed with short periods of deep bradycardia. Night-time recording revealed hypoxemia (mean oxygen saturation of 85.4%), and cardio-respiratory monitoring disclosed a severe central apnea. An EEG showed slowing of the background rhythm. Intravenous high dose steroids were administered without benefit. Over the following months, the patient presented recurrent hypoxic events, and eventually died 15 months from the disease onset during an attack characterized by extreme bradycardia.

**Neuropathology**

The brain was obtained at necropsy in Padua and selected tissue samples from the right cerebellar and the right cerebral hemisphere, including thalamic nuclei, were immediately frozen and kept at −80°C until molecular analyses. The rest of brain was fixed in formalin and thereafter processed for histopathological studies at the Neuropathology Laboratory in Verona.

Formalin-fixed brain was sliced coronally at 1 cm intervals. Tissue blocks were obtained from frontal, temporal, parietal and occipital cortices, hippocampus, basal ganglia, thalamus, amygdala, nucleus basalis, hypothalamus (including anterior hypothalamic area, tuberal region and mamillary region), brainstem, and cerebellum. Paraffin sections were stained with hematoxylin and eosin (H&E). Immunohistochemistry was undertaken using the avidin-biotin-peroxidase complex (Vectastain ABC Elite kit, Vector Laboratories) with the following primary antibodies: anti-PHF-tau antibody (clone AT8; Innogenetics, Ghent, Belgium), anti-RD4 anti-
body (clone 1E1/A6; Merck Millipore, Darmstadt, Germany), anti-RD3 antibody (clone 8E6/C11; Merck Millipore), anti-GFAP (clone 6E2; Dako, Glostrup, Denmark), anti-amyloid-β (clone 6F/3D; Dako), anti-α-synuclein (clone LB509; Invitrogen, Carlsbad, CA, USA), anti-ubiquitin (polyclonal; Dako), anti-TDP-43 (clone pS409/410; Cosmo Bio Co. Ltd, Tokyo, Japan), anti-CD68 (clone PG-M1; Dako), anti-CD163 (clone 10D6; Novocastra, Newcasle, UK), anti-CD11c (clone 5D11; Novocastra), anti-pSTAT (polyclonal; Santa Cruz Biotechnology, Dallas, TX, USA), anti-Iba1 (polyclonal; Wako Chemical, Richmond, VA, USA), anti-CD4 (clone 1E1/A6; Merck Millipore), anti-amyloid-β (clone LB509; Invitrogen, Carlsbad, CA, USA), anti-

**TDP-43 selective fractionation and western blot**

Frozen brain samples from the index case and three controls, including two subjects with stroke and one with multiple sclerosis, were homogenized in ten volumes of TBS (50 mM Tris-HCl, pH 7.5, 150 mM NaCl), 10% sucrose, phosphatase inhibitor cocktail (Roche) and centrifuged at 200,000 g for 1 h at 4°C. The supernatant was recovered as the Tris-soluble fraction (S1). The Tris-insoluble pellets were lysed in the same buffer containing 1% (v/v) Triton X-100. The homogenates were left at 37°C for 30 min and then centrifuged at 200,000 g at 4°C for 1 h. The resultant supernatant was retained as a Triton-soluble fraction (S2) and the pellet was homogenized in TBS buffer, 10% sucrose, phosphatase inhibitor cocktail (Roche) containing 1% sarkosyl. The homogenate was incubated at 37°C for 30 min and then subjected to a 1 h spin at 200,000 g. The resultant supernatant was retained as the sarkosyl-soluble fraction (S3), and the pellet was solubilized in one volume of 50 mM Tris–HCl, pH 7.5, containing 8 M urea. This process was followed by a 1 h centrifugation at 200,000 g. The supernatant was retained as the sarkosyl-insoluble, urea-soluble fraction (P). The soluble and sarkosyl-insoluble fractions were solubilized in SDS-sample buffer and loaded on 13% SDS-PAGE gels. Proteins were transferred onto PVDF membrane (Immobilon P, Millipore) for 2 h at 60 V, and then incubated overnight at 4°C with anti-TDP-43 antibody (Proteintech, TDP-43 Polyclonal Antibody Cat.n. 10782-2-AP). Blots were developed with an enhanced chemiluminescence system (ECL, Amersham Biosciences) and visualized on autoradiographic films (Hyperfilm, Amersham Biosciences).

**RESULTS**

**Neuropathology**

Macroscopic examination of the brain was unremarkable. Microscopic studies revealed only rare perivascular lymphocytic infiltrates (Fig. 1A, B) of CD4+ and CD8+ T cells and CD20+ B cells, but not CD138+ plasma cells, in the thalamus, basal ganglia, and midbrain (Fig. 1C). Mild neuronal loss and reactive astrogliosis were observed in the thalamus, fronto-basal cortex, nucleus basalis, amygdala, and locus coeruleus (Fig. 1D–F). Immunohistochemistry with AT8 antibody revealed the presence of tangles in the nucleus basalis (Fig. 1G), and amygdala (Fig. 1H), but not neocortical areas, basal ganglia, and hypothalamus, in standard sections harvested from tissue blocks obtained at 10 mm intervals from the optic chiasm to mammillary bodies. However, at variance with earlier results, examination of multiple serial sections of the hypothalamus showed the presence of focal areas of AT8-immunoreactivity, with prevailing neurit threads and sparse neuronal tangles, in dorsomedial and ventromedial nuclei. AT8-positive tangles were also seen in the locus coeruleus (Fig. 1I), being composed of a mixture of 3R and 4R isoforms, but no tau pathology was observed in other adjacent nuclei of pontine tegmentum nor in other brainstem tegmental nuclei. AT8, anti-RD3 and RD4 tau antibodies showed neurofibrillary tangles (NFTs), pretangles, and neurit threads in all hippocampal subregions, especially in CA2 sector, and entorhinal cortex (Fig. 1J, K); no involvement of dentate gyrus was observed. Several tangles in the hippocampus were ubiquitin-positive (Fig. 1L), a finding suggestive of tau truncation at Asp421, a proteolytic event occurring at early/intermediate stages of NFT maturation [2]. No accompanying extracellular deposition of amyloid-β was seen throughout all investigating sections and no α-synuclein aggregates were detected. Immunohistochemistry with anti-phosphorylated TDP-43 antibody revealed neuronal skein-like and granular cytoplasmic inclusions in the thalamus, striatum, and midbrain, findings consistent with both advanced and early maturation stages of TDP-43 aggregation [3], in addition to occasional oligodendroglial TDP-43 pathology (Fig. 2A, B). Surprisingly, TDP-43 positivity was also observed in parenchymal ramified microglial cells and in...
perivascular microglia, in the thalamus, hippocampal complex, striatum, nucleus basalis, and midbrain (Fig. 2C-F). Activated microglia highly expressed CD11c, CD68, and to a lesser extent pSTAT1 and Iba1; at variance, anti-CD163 immunoreactivity was detected in elongated perivascular macrophages and in clusters of poorly ramified parenchymal cells (Fig. 3A-D). These findings are overall suggestive of the involvement of distinct microglial phenotypes and populations, including resident and blood-derived cells.

Biochemical characterization of TDP-43

Immunoblot analysis with anti-N-terminal TDP-43 polyclonal antibody of brain homogenates from the thalamus of the index case and from three control cases, showed that abnormal TDP-43 molecular
Fig. 2. Microglial and neuronal TDP-43 pathology. Immunohistochemistry with anti–phosphorylated TDP-43 showing oligodendroglial pathology (A, arrows), skein-like (A, arrowhead) and granular neuronal cytoplasmic inclusions (B) in the thalamus. TDP-43 immunolabeling of activated parenchymal and perivascular microglial cells in the thalamus (C), mesencephalon (D), nucleus basalis (E), and pallidum (F). Western blot of Tris-soluble (S1), Triton-soluble (S2), sarkosyl-soluble (S3) and sarkosyl-insoluble, urea-soluble (P) fractions of brain homogenates from the index case and a control subject showing the expression of 35-kDa and 25-kDa TDP-43 truncated fragments (G, TDP-43 detected with N-terminal TDP-43 polyclonal antibody, Proteintech, Cat. N. 10782-2-AP). Scale bars: A-H 50 μm.

Species and abundant Sarkosyl-insoluble TDP-43 were recovered in brain samples from IgLON5-related tauopathy, but not from control tissues. In particular, using a phosphorylation-independent anti-TDP-43 antibody, aberrant TDP-43 species, including truncated fragments of 35-kDa and 25-kDa,
Fig. 3. Representative regions involved by diffuse and focal microglial activation. Activated ramified and perivascular microglial cells are observed in the hippocampus (A), thalamus (B, C), and nucleus basalis (D) with anti-CD11c (A, B), anti-CD68 (C), and anti-CD163 (D) antibodies. Scale bars: A-F 50 μm.

were detected in Tris-soluble and Sarkosyl-insoluble fractions (Fig. 2G). Moreover, as observed in different immunoblot replicates, the Tris-soluble fraction contained also additional abnormal lower-molecular-weight TDP-43 species, migrating in an 18–25 kDa zone, which were barely detected in the Sarkosyl-insoluble fraction. On the contrary, the latter fraction contained large amounts of higher Mr TDP-43 aggregates, producing a smear spanning from the top of the gel to 25 kDa. Collectively, these results confirm the presence of heterogeneous pathological TDP-43 species which are mostly retrieved in the insoluble fraction.

DISCUSSION

The present study discloses the co-existence of neuronal tau pathology and microglia/neuronal TDP-43 pathology in a subject with autoimmunity to IgLON5, who presented a fatal progressive neurological syndrome characterized by neuropsychiatric manifestations, sleep disorder, dysautonomia, and cognitive decline. While clinical manifestations in this case are consistent with the anti-IgLON5 syndrome, the neuropathological phenotype shows some divergences with that reported in the six cases so far investigated, including three subjects with known antibody status and three cases with a typical clinicopathological phenotype but unknown antibody status [4]. Indeed, in the index case, the regional distribution of tau pathology is at variance with the expected diffuse hypothalamic/brainstem involvement with increasing cranio-caudal severity, but rather shows an AD-like pattern, due to the isolated involvement of the locus coeruleus, but not other brainstem nuclei, in addition to tau deposition in the hippocampus, entorhinal cortex, and amygdala; on the contrary, similarities consisted in the presence of tau
aggregates in hypothalamic nuclei of the tuberal region, and in the lack of concomitant deposition of amyloid-β. Although the duration of the disease in postmortem examined cases ranges from 6 months to 13 years [1, 4], an additional divergent feature is the occurrence of rapidly evolving cognitive decline in the index case, which is in contrast with the absence of cognitive impairment reported in the aforementioned neuropathologically investigated cases, despite the detection of severe tau pathology in the hippocampal complex [4].

The most relevant feature of the present study regards the detection of TDP-43 pathology with a prevailing burden in regions not affected by tau pathology, such as basal ganglia, thalamus, nucleus basalis, and midbrain. These findings suggest an independent origin and eventual spreading of these two proteinopathies, which is at variance with the pattern observed in neurodegenerative conditions where tau and TDP-43 pathology coexist [5]. Intriguingly, TDP-43 pathology was mostly detected in microglial cells, being characterized by cytoplasmic mislocalization of abnormal phosphorylated and fragmented neurotoxic isoforms [6–8]. To our knowledge, this observation is unprecedented in human pathology, although there is consistent evidence to support a major role for TDP-43-overexpressing microglia in promoting neuronal death both in experimental cellular and animal models of amyotrophic lateral sclerosis [9–11]. While our findings warrant cautious interpretation, our data suggest a pathogenic role for TDP-43 in anti-IgLON5-related tauopathy through a microglia-mediated non-cell autonomous neuronal death, or, in alternative, the consequence of anti-IgLON5-induced neurodegeneration. Although the precise role of autoimmunity to IgLON5 remains to be determined, the symptomatic remission with intravenous immunoglobulin [12] or antibody clearance via plasmapheresis combined with glucocorticoid therapy [13] observed in some patients, suggests the occurrence of antibody-mediated pathogenic mechanisms.

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SUPPLEMENTARY MATERIAL

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REFERENCES


