Case report

Stiff person syndrome associated with lower motor neuron disease and infiltration of cytotoxic T cells in the spinal cord

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A B S T R A C T

We present a 67-year-old non-diabetic male who presented with muscle cramps, paresis, atrophy and fasciculations in the left leg, followed by rapidly progressive muscle stiffness and superimposed spasms which subsequently also affected the right leg and the trunk. GAD65 autoantibodies were elevated in serum and CSF, compatible with systemic and intrathecal synthesis of oligoclonal and high-avidity autoantibodies, and GAD65 specific T cells were clonally expanded in the CSF. The patient did not respond to GABAergic and immunomodulatory treatment or plasma exchange, and died from respiratory failure after 18 months. Autopsy revealed unilateral axonal swelling, chromatolysis and vacuolisation of anterior horn cells of the lower spinal cord, accompanied by microglia proliferation and discrete infiltration of CD8+ cytotoxic T cells. No CD4+ T helper cells, B cells or complement deposition were detected. To our knowledge, this is the first report of stiff person syndrome with lower motor signs restricted to a lower limb, and also the first attempt to characterize the infiltrating T cells. The finding of CD8+ cytotoxic T cells in the absence of B cells in the inflamed area of the spinal cord suggests that the intrathecal synthesis of GAD65 autoantibodies takes place in areas of the CNS not strictly related to the clinically relevant lesions.

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1. Introduction

Stiff person syndrome (SPS) characterized by muscular rigidity and superimposed spasms has been known for more than 50 years [1]. The spectrum of SPS-related disorders also comprises the more localized stiff limb subtype [2], the jerking man syndrome [3], and progressive encephalomyelitis with rigidity (PER) [4]. The frequent finding of autoantibodies against glutamic acid decarboxylase (GAD) 65 and the association with other autoimmune diseases suggest an immune mediated mechanism [5,6]. Accordingly, transfer of serum induces a SPS-like phenotype in rats [7], but it is not clear if or how these antibodies reach GAD65 within the cytoplasm of neurons. More knowledge of the pathology related to clinical expressions of SPS is therefore warranted.

We have previously reported that the GAD65 autoantibodies in CSF and serum are oligoclonal and have high binding avidity [8]. One patient with substantial intrathecal production of oligoclonal GAD65 autoantibodies and clonal expansion of GAD65 specific CD4+ T cells [9] (patient SPS 3 in the original reports) has deceased, thereby allowing combined interpretation of clinical, immunological and histological data. The symptoms included both stiffness and spasms typical of SPS as well as lower motor signs, and autopsy revealed unilateral infiltration of cytotoxic T cells and microglia activation in the anterior horn of the lower part of the spinal cord. We discuss the potential pathogenic relevance of these findings for the clinical symptoms and signs in this patient.

2. Case history

A previously healthy 67-year-old male presented with rapidly evolving and painful flexor cramps of the toes on his left foot, followed by fasciculations and muscle atrophy in the left leg. The condition progressed rapidly with marked muscular rigidity and painful superimposed spasms. This was most prominent in the left leg, but subsequently also involved the right limb and truncus leading to frequent falls and immobilisation. Neurological examination 7 months after symptom debut revealed generalized atrophy in the left leg with paralysis of the left ankle. Muscle stiffness was prominent in the left leg but was also found in the right leg and the trunk, corresponding to a stiffness score of five out of six possible points at the SPS stiffness extent scale [10]. Moreover, auditory, somatosensory, emotional and visual stimuli and attempts to move the left leg triggered painful muscle cramps, corresponding to six out of seven...
possible points at the SPS heightened sensitivity scale [10]. The tendon reflexes were absent in the left leg, weak in the right leg, and normal in both arms. Babinski sign was negative on the right side, and indifferent on the left.

Electromyography of the left leg 10, 13, 14 and 16 months after symptom onset showed continuous motor activity and signs of denervation with positive sharp waves. At month 10 after symptom onset, routine blood tests including glucose and electrolytes, extensive radiological examinations of the neuraxis, malignancy workup, antibodies against gangliosides, voltage gated potassium channels, pancreas islets, gephyrin, amphiphysin, DNA and thyroxin peroxidase were all normal. Serum creatinine increased gradually to 1717 units/ml (normal <270 U/ml), but normalized 3 months later. Routine examinations of the cerebrospinal fluid (CSF), including isoelectric focusing, cell count and quantification of albumin, protein and IgG, as well as the ratio of IgG:albumin in CSF:serum were normal. Anti-GAD65 antibodies were markedly and persistently increased in serum (0.96–1.57 units at month 10 and 14 after symptom onset, respectively; normal <0.08) and were also detected in CSF (0.84 units at month 10 after symptom onset). The CSF:serum relative ratio of anti-GAD65 antibodies was 3.2 (normal <1.3), and isoelectric focusing-immunoblot displayed several GAD65 specific oligoclonal IgG bands in CSF with no or clearly weaker counterparts in serum [8].

Intravenous diazepam and clonazepam induced transient improvement, but there was no persistent response to these drugs or to baclofen, phenytoin and intravenous immunoglobulins. The patient deteriorated rapidly and became bedridden with almost continuous painful spasms in the left leg. Plasma exchange was therefore performed 14 months after symptom onset with removal of 31 of plasma at five subsequent days. The serum and CSF GAD65 antibody activity was 1.57 and 0.84, respectively, prior to plasma exchange, and 1.28 and 0.57 after the last procedure. The anti-GAD65 activity in the consecutive plasmapherases was 1.25, 1.28, 1.34, 1.15 and 1.19 units, respectively. The patient did not respond to plasma exchange. The procedure was complicated by transient lymph leakage and catheter pain, and was therefore not repeated. Two g/kg intravenous immunoglobulin was given after another month, but did not affect the increasing rigidity and painful muscle spasms. He died from pneumonia 18 months after symptom onset. No signs of malignancy were detected at autopsy.

3. Neuropathological examination

After fixation in 10% formalin, brain, spinal cord and a fragment of the sciatic nerve were embedded in paraffin wax. Five μm thick sections were cut and stained with haematoxylin and eosin (HE). Sections from selected areas of the brain and five segments (cervical, thoracic, lumbar, sacral and conus medullaris/cauda equina) of the spinal cord were stained with the Bodian, Gallyas, Campbell-Switzer and Luxol fast blue techniques. Immunohistochemical analyses were performed using primary antibodies against ubiquitin (Dako), glial fibrillary acidic protein (Dako), alfa-synuclein (Zymed), tau (Dako), the leukocyte common antigen (LCA) CD45 (Dako), CD3 (NeoMarkers), CD4 (Novocastra), CD8 (Dako), CD20 (Dako), CD79a (Dako), CD68 (Dako), the complement complex 4d (C4d; Biomedica) and terminal complement complex C3C (Dako), and immunoglobulines A, G and M (Southern Biotechnology Associates).

The brain contained senile plaques throughout the cortex but few neurofibrillary tangles (Braak stage 1–2). Except from a few axonal swellings in the granular cell layer of the cerebellum no other structural changes were detected in the brain. Examination of the spinal cord showed chromatolysis and vacuolisation of some anterior horn cells, as well as axonal swellings and slight gliosis. These changes were more pronounced in the lower segments (Fig. 1). Ubiquitin-immunoreactive neuronal inclusions consistent with amyotrophic lateral sclerosis were not detected. A discrete, distinctively unilateral accumulation of CD8+ cytotoxic T cells and proliferation of CD68+ microglial cells in the anterior horn was detected by immunohistochemical examination (Fig. 2). There were no signs of inflammation elsewhere. Deposition of the complement complexes was not detected. The spinal nerve roots were normal, whereas a slight loss of nerve fibres were detected in the sciatic nerve. A snap frozen biopsy from the vastus lateralis muscle demonstrated signs of denervation and no evidence of inflammation.

4. Discussion

The combination of lower motor neuron involvement in one leg with paresis and fasciculations combined with muscular rigidity and superimposed spasms add to the clinical heterogeneity of SPS-related disorders. Selective involvement of the lower limbs is typical for stiff limb syndrome [2]. The prominent lower motor neuron signs and the relentless and progressive course that led to death after 18 months is not typical for SPS, but rather points to a diagnosis of PER. PER is distinguished from SPS by additional brainstem or long tract signs, CSF pleocytosis, and a more aggressive course with inferior response to GABAergic or immunomodulatory treatment [11–15]. Lower motor neuron signs in the upper limbs have been reported in PER [16,17], but to our knowledge this is the first
Fig. 2. Unilateral inflammation of the anterior horn in the lower spinal cord. Staining with the leukocyte common antigen CD45 revealed unilateral accumulation of leukocytes in the lower segments of the spinal cord (dark brown; upper panel). The leukocytes stained positive with antibodies against the pan-T cell marker CD3 and the cytotoxic T cell marker CD8 (dark brown; middle panels), but were negative for the T helper cell marker CD4 and the B cell markers CD20 and CD79a (data not shown), and were therefore CD8+ cytotoxic T cells. Microglia cells in the same area of the spinal cord stained positive with anti-CD68 antibody (dark brown; lower panels). Original magnification 40× (A, C and D), and 20× (B).
report of predominant lower motor involvement in the lower limb. The patient also suffered typical symptoms of SPS with tonically maintained rigidity and muscle cramps, supporting the relevance of the concept “SPS plus” syndromes [16].

The immunopathological findings comprised intrathecal synthesis of high-avidity oligoclonal IgG antibodies against GAD65 [8], evidence of clonally expanded GAD65-specific CD4+ T cells restricted by DRB1*0801 [9], and infiltration of cytotoxic CD8+ T cells and microglia activation in the spinal cord. Lymphocyte cuffing of small CNS vessels is a typical finding in PER [12–14]. Similar findings have been reported in some cases with typical SPS [12,14,18–21], whereas others have not found evidence of inflammation [22,23]. Vacular degeneration of anterior horn cells at the lumbar segments of the spinal cord associated with prominent microglia proliferation has previously been described in a patient with atypical SPS [24]. T cell infiltration and microglia activation may play a primary pathogenic role, but could also be secondary to neuronal damage caused by other factors. Thus, T cell infiltration and microglia activation is observed also in degenerative diseases like amyotrophic lateral sclerosis and its animal model [25]. This distinction has therapeutic implications, because aggressive anti-inflammatory treatment would hardly be beneficial unless the inflammation is pathogenic. CD8+ cytotoxic T cells may recognize antigen presented on HLA class I molecules by neurons [26], and are believed to be important effector cells in inflammatory CNS disorders [27]. HLA class I expression has not been studied in SPS or PER, but has been demonstrated on neurons in both inflammatory and degenerative CNS diseases [28]. It is therefore conceivable that infiltrating T cells could contribute to the irreversible and destructive changes observed in some patients with SPS-related disease, including the focal neurodegeneration observed in our patient. However, although the co-localization of inflammation and lower motor neuron involvement makes it tempting to speculate on a causal relationship, it should be emphasized that the T cell infiltration was more discrete than usually observed in inflammatory CNS disorders.

The intrathecal synthesis of oligoclonal antibodies and the evidence of clonally expanded HLA class II restricted T cells specific for GAD65 previously demonstrated in this patient implies intrathecal persistence of B cell clones producing these antibodies [8,9]. The lack of B cells, plasma cells and T helper cells within the inflamed section of the spinal cord suggests that the intrathecal synthesis of GAD65 antibodies takes place elsewhere, either in the CNS or in the meninges. This needs to be investigated in a larger sample set, but may indicate that the intrathecal synthesis of GAD65 autoantibodies is not directly related to the tissue destruction.

It is expected that five plasma exchanges removing 3 l of plasma each would deplete at least 90% of the serum autoantibodies. However, plasma exchange did only induce a discrete drop in the GAD65 antibody activity in serum, plasmapherates and CSF, and did not induce clinical improvement in our patient. This may appear as a paradox, as 151 of plasma with high GAD65 activity was removed from the patient. The persistence of GAD65 antibody activity in serum and CSF may reflect that the radioimmunoassay is relatively insensitive to changes in GAD65 antibody levels at this order of magnitude, or that GAD65 antibodies depleted from serum is replaced from tissues and extracellular fluid. We can therefore not exclude that more aggressive removal of autoantibodies could have been helpful, although this seems unlikely given the signs of neurodegeneration detected shortly afterwards.

The etiology of SPS is enigmatic and most likely multifactorial. SPS has been reported after infection with West Nile virus [29], and T cells may cross-recognize GAD65 and microbial proteins [30]. Our patient had no previous history of serious infections or exposure to toxins. There was no family history of SPS-related disorders, but he did carry the HLA-DRB1*0301 allele reported to be associated with autoimmune diseases including SPS [10]. This allele is, however, carried by almost one third of Norwegians. The GAD65 specific T cells cloned from the CSF of this patient was not restricted by HLA-DRB1*0301 [9], and the significance of HLA-DRB1*0301 in this patient should be interpreted with care.

Conflicts of interest

We have no conflicts of interest.

References