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Case report

Paraneoplastic intestinal pseudo-obstruction associated with high titres of Hu autoantibodies

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Abstract. Anti-Hu autoantibodies in high titres, as revealed with immunocytochemistry and Western blot, were present in a patient with gastrointestinal pseudo-obstruction and small-cell lung cancer (SCLC) bearing the Hu antigen. Marked neuron and nerve fibre loss were found in the myenteric plexus at postmortem. These findings show that neuronopathic Hu-associated gastrointestinal pseudo-obstruction can occur as the only paraneoplastic neurological symptom in patients with SCLC.

Key words: Small-cell lung cancer – Paraneoplastic encephalomyelitis/sensory neuronopathy – Pseudo-obstruction – Visceral neuronopathy – Hu antigen/antibody

Introduction

Chronic intestinal pseudo-obstruction is a well-recognized paraneoplastic syndrome associated with small-cell lung cancer (SCLC) (Schuffer et al. 1983; Camilleri et al. 1985; Chinn and Schuffer 1988; Lennon et al. 1991). The syndrome is due to damage of neurons of the enteric neural plexus by still unknown pathogenic mechanisms.

Patients with paraneoplastic encephalomyelitis or paraneoplastic sensory neuronopathy (PEM/PSN) and SCLC harbour an autoantibody called anti-Hu (Graus et al. 1985; Dalmau et al. 1992b). High titres of anti-Hu autoantibodies are highly restricted to patients with PEM/PSN (Dalmau et al. 1990). Although autonomic dysfunction and neuronal loss in the enteric plexus may be seen as part of the PEM/PSN syndrome, paraneoplastic intestinal pseudo-obstruction has not been considered as part of the different neurological syndromes included under the term PEM/PSN.

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We report on a patient with paraneoplastic gastrointestinal pseudo-obstruction associated with high titres of anti-Hu as the sole clinical manifestation of an SCLC.

Case report

A 61-year-old man was admitted in 1988 complaining of abdominal pain, weight loss, severe constipation, abdominal distension and vomiting. Radiographic studies showed dilatation of the stomach, small intestine and colon. Barium enema, colonscopy and CT examination did not reveal the presence of tumour. A diagnosis of gastrointestinal pseudo-obstruction was made, but no additional studies were conducted. The patient was treated with enemas at the time of admission and during the following 3 years. During this time he lost 20 kg. Repeated radiographic studies disclosed a progressive increase in the intensity of the gastrointestinal distension.

At the beginning of 1992 he presented with right hemiparesis A cranial CT scan showed a large space-occupying mass in the left parietal lobe. Chest radiography revealed an enlargement of the left hilum. In retrospect, a left hilar prominence, although unnoticed at that time, was already present in the chest radiograph of 1988. The patient also had a subcutaneous nodule of 2 cm in diameter in the left submammary region, which, on fine-needle aspiration biopsy, proved to be undifferentiated small-cell carcinoma. The patient died of bronchopneumonia 15 days after admission.

At autopsy, a SCLC was found in the left hilum with systemic metastases involving the regional lymph nodes, adrenal glands and kidneys, and the left parietal lobe. Metastases were not found in the peritoneal cavity, gastrointestinal tract and related sympathetic ganglia. No other abnormalities were seen in the central nervous system. The Hu antigen was demonstrated in the primary tumour with immunocytochemistry using a purified human biotinylated Hu antibody at a dilution of 1:1000 (Fig. 1). This serum was obtained from a patient, de-

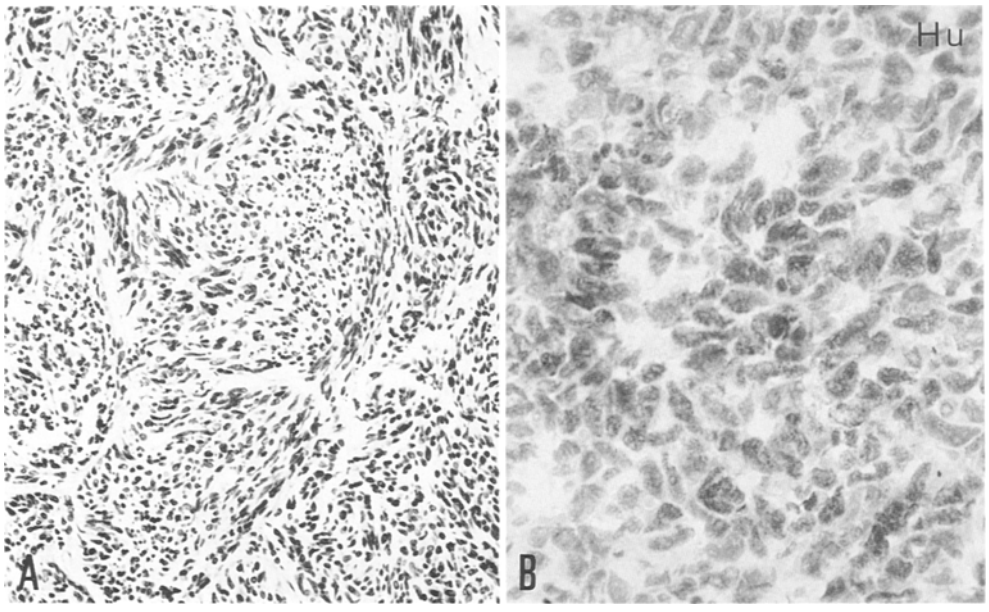

Fig. 1A, B. The primitive tumour: small-cell lung carcinoma (SCLC) (**A**) is immunoreactive with a purified anti-Hu biotinylated IgG (**B**). **A** Haematoxylin and eosin ×160; **B** frozen section with no counterstaining. ×1000

scribed elsewhere (Graus et al. 1990), who had suffered from paraneoplastic encephalomyelopathy with no known gastrointestinal pathology. The serum of this patient recognized protein bands of 35–40 kDa molecular weight by Western blot, and stained neurons of the central and peripheral nervous system by immunocytochemistry. The specificity of the antibody was tested by incubating a few tissue sections without the primary serum (negative staining), and demonstrating abolition of immunoreactivity by pre-incubation with a different non-biotinylated anti-Hu positive serum.

The whole gastrointestinal tract of the present case was severely distended, and, on microscopic examination, there was marked loss of neurons and fibres in the myenteric plexus as revealed in routine sections and with S-100 and neuron-specific enolase (NSE) immunocytochemistry (Fig. 2). Inflammatory infiltrates were not seen in the enteric plexus. The muscular layer was normal.

Serum obtained premortem was examined for the presence of Hu autoantibodies with immunocytochemistry and Western blotting. For immunocytochemistry, dewaxed paraffin sections of rat cerebellum and spinal ganglia, fixed in Carnoy for 6 h, were incubated overnight at 4° C with the serum of the patient at a dilution of 1:500 in phosphate-buffer saline (PBS); this was followed by rabbit anti-human IgG, and peroxidase-anti-peroxidase complex. The peroxidase reaction was visualized with 0.05% diaminobenzidine and 0.01% hydrogen peroxide. The serum of the patient, but not the serum of a control, reacted with central and peripheral neurons (Fig. 3). Sections incubated without the primary antibody were negative. On frozen sections of normal human frontal cortex, the serum immunoreacted with the neuronal nuclei at dilutions higher than 1:5000 using the avidin-biotin-immunoperoxidase technique. Western blot analysis with extracts of cortical neurons, done as previously described (Dalmau et al. 1990), showed that the serum of the patient recognized protein bands of 35–40 kDa molecular weight (Fig. 4).

Discussion

This patient had a paraneoplastic chronic intestinal pseudo-obstruction as the sole clinical manifestation of an SCLC, and high titres of circulating anti-Hu autoantibodies, as revealed with immunocytochemistry and immunoblotting. The absence of inflammatory changes in the myenteric plexus can be attributed to the long time that had elapsed since the onset of the presumed autoimmune reaction.

Although low titres of anti-Hu antibodies are detected in 16% of patients with SCLC without paraneoplastic syndromes, the finding of high titres of anti-Hu autoantibodies in this patient suggests that they may be relevant in the pathogenesis of the paraneoplastic syndrome rather than be a simple marker of the presence of a SCLC. Anti-neuronal nuclear autoantibodies, which reacted with neurons of the myenteric plexus have been found in four patients with chronic pseudo-obstruction and SCLC (Lennon et al. 1991). Although immunoblotting was not done in those cases, these antibodies are probably the same as the anti-Hu since they show a similar pattern of reactivity (Altermatt et al. 1991; Graus and Dalmau 1991).

It is noteworthy that 4 years elapsed from the first gastrointestinal symptoms to the diagnosis in the present case. At first glance it appears to be a very long time for a SCLC to remain undetected. In previously reported cases of PEM/PSN and paraneoplastic intestinal pseudo-obstruction the median time between the development of neurological symptoms and the diagnosis of the tumour was 4 months (Dalmau et al. 1992b), ranging from 1 to 26 months (Camilleri et al. 1985; Chinn and Schuffler 1988; Lennon et al. 1991). However, as already

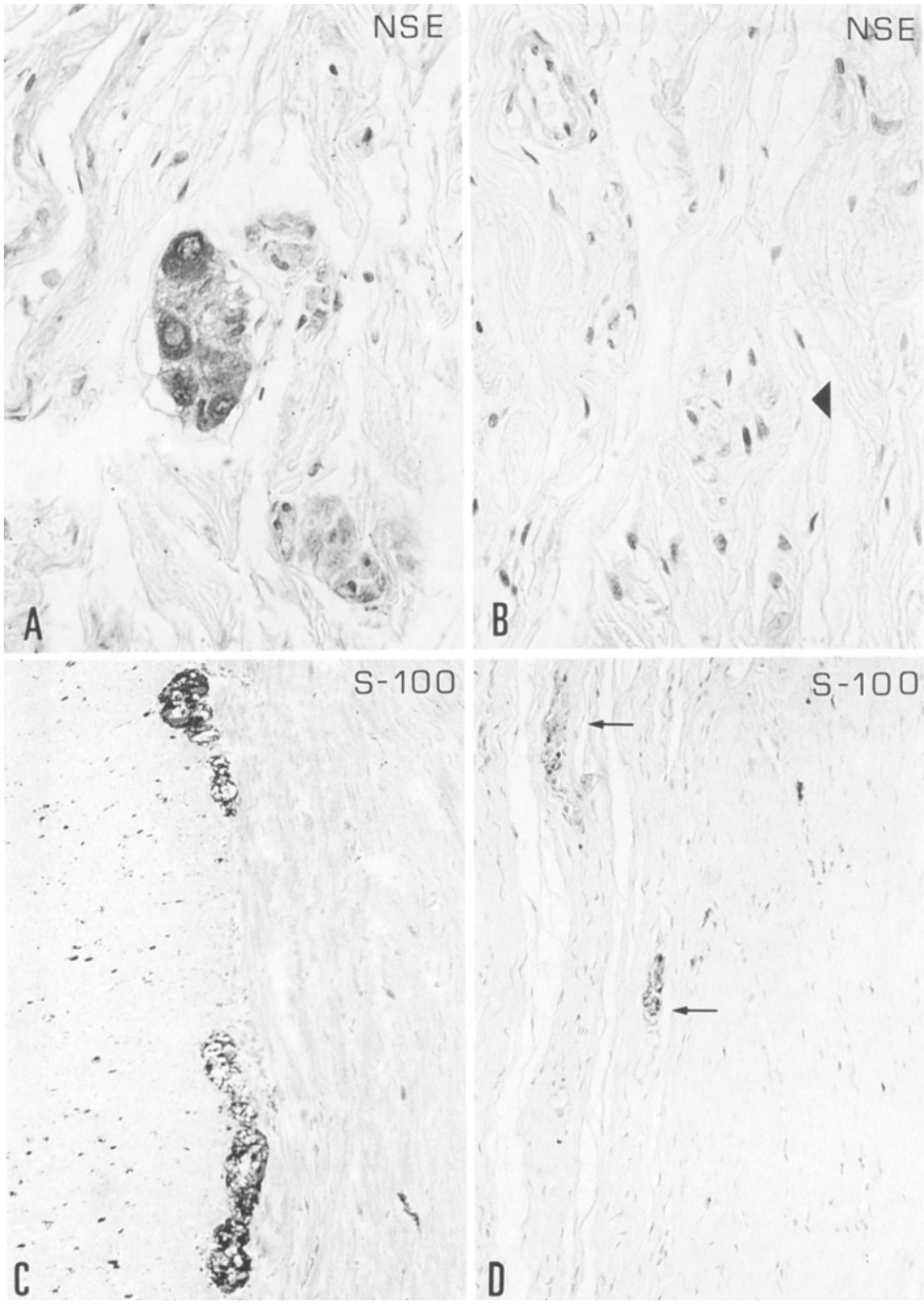

Fig. 2A–D. Myenteric plexus of a control (**A** and **C**) compared with the patient (**B** and **D**) processed for neuron-specific enolase (NSE) and S-100 immunocytochemistry. Marked reduction of ganglion cells (**A** and **B**, *arrowhead*), and fibres (**C** and **D**, *arrows*) is found in the myenteric plexus of the patient. **A** and **B** ×160; **C** and **D** ×100

mentioned, an enlarged left hilum was present at the beginning of the disease. Therefore, it is possible that the tumour was present for 4 years. Consistent with this, it has been shown that SCLC associated with high titres of anti-Hu appear to be smaller at diagnosis, and to run a less progressive clinical course, than SCLC lacking an antibody response (Dalmau et al. 1992b). Furthermore, SCLC may even regress in patients with paraneoplastic neuronal antibodies (Darnell and DeAngelis 1993).

The reasons of the production of the Hu antigen by SCLC are not clearly known. In normal tissues the Hu antigen is highly restricted to the nervous system. In lung tumours, the Hu antigen is found in in all SCLC. Although the neuroendocrine origin of SCLC is widely accepted, only 50% of neuroblastomas, and few other neuroendocrine tumours express the anti-Hu antigen (Dalmau et al. 1992a).

Patients with paraneoplastic gastrointestinal pseudo-obstruction sometimes present with signs of vegetative dysfunction or sensory neuropathy (Ahmed and Carpenter 1975; Lhermitte et al. 1980; Schuffler et al. 1983). The finding in these patients of the anti-Hu antibody in high titres, a feature not usually seen in paraneoplastic

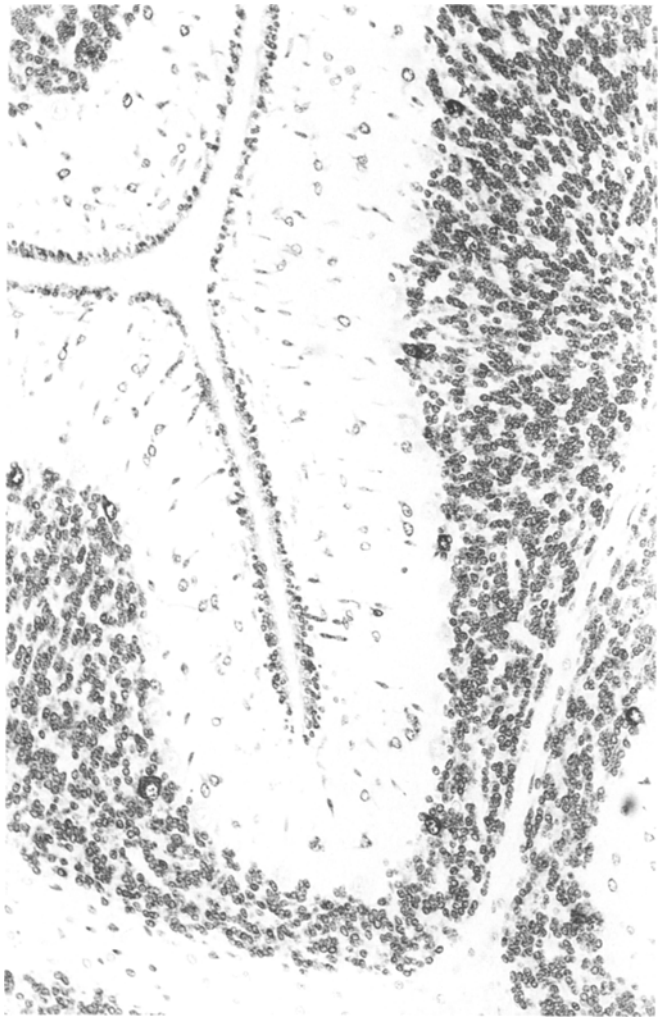

Fig. 3. Immunocytochemistry with the serum of the patient and normal rat cerebellum. All the cerebellar neurons are immunoreactive with the serum. ×160

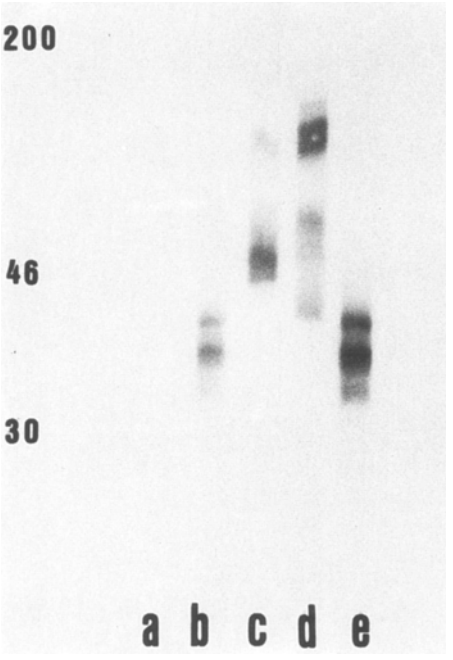

Fig. 4a–e. Western blot of cortical neurons extract probed with **a** normal human serum, **b** anti-Hu positive serum, **c** anti-Ri positive serum, **d** serum with an anticytoplasmic antineuronal antibody, and **e** serum of the patient. The latter recognizes the same bands identified with the anti-Hu positive serum

neurological syndromes other than PEM/PSN, suggests that, at least, some cases of paraneoplastic intestinal pseudo-obstruction could share with PSN and the different neurological syndromes included in PEM similar pathogenic autoimmune mechanisms.

Finally, the present study confirms that Hu immunocytochemistry is a useful and sensitive tool in the diagnostic approach of Hu-associated paraneoplastic syndromes. Although the antigen is very vulnerable to different fixative solutions, Carnoy fixation for about 6 h, followed by paraffin-embedding permits a good visualization of the antigen.

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