

Scientific correspondence

Neocortical neurones may be targeted by immune attack in anti-Yo paraneoplastic syndrome

Paraneoplastic neurological disorders are immune-mediated, and not caused by direct effects of the tumour, metastases, cancer induced coagulopathies, metabolic and nutritional deficits, infections or side effects of therapy [1]. They often take the patient to the doctor months before the underlying neoplasm is identified. Antineuronal antibodies react with both the nervous system and the underlying cancer [1]. Their detection in patients' sera or cerebrospinal fluid may establish an early diagnosis, and focuses the search for a tumour on to a limited number of organs [1,2]. It has been reported that in anti-Yo PCD (paraneoplastic cerebellar degeneration) humoral and cytotoxic immune responses were exclusively directed against Purkinje cells and brainstem neurones [2–5]. Almost always, the patients are elderly women who present with limb and truncal ataxia, often along with dysarthria. The underlying neoplasms are gynaecological cancers (ovary, endometrium, fallopian tube) or breast cancer. Until now, merely anecdotal reports of anti-Yo paraneoplastic disorder in men exist. In these, adenocarcinomas of the oesophagus [6,7], gastric adenocarcinoma [8], parotid adenocarcinoma [9] and prostatic adenocarcinomas [10] were found. Here, we report a male patient with an adenocarcinoma of the lung and positive for anti-Yo, who developed PCD and aphasia. In addition to already well-described Purkinje cell loss and cortical degeneration of the cerebellum [3,4], our data suggest that other neurones in the cerebral hemispheres may serve as targets in anti-Yo associated immune attack.

A 66-year-old right-handed man presented with a 1-week history of progressive gait ataxia, acute vertigo and incipient slurred speech and language disturbance. Four weeks before admission, a routine chest X-ray examination showed hilar lymphadenopathy. The following biopsy of enlarged bronchial lymph nodes revealed invasive nonsmall cell lung cancer cells. Apart from smoking heavily for more than 40 years, the patient's history was

completely unremarkable. On physical examination, the patient appeared well but was slightly overweight (74 kg/170 cm height). The temperature was 36.8°C, pulse was 75 beats per minute, blood pressure was 130/85 mm Hg, and respiratory rate was 15 breaths per minute. Heart, lungs and abdominal examination were normal. There was no palpable lymphadenopathy, peripheral oedema or digital clubbing. An electrocardiogram showed sinus rhythm at rate of 80 per minute, and normal ST- and T-waves.

On initial neurological examination, the patient was alert and orientated. He spoke with moderate cerebellar dysarthria. Comprehension and naming were normal. Moderate cerebellar gait ataxia was present. Computed tomography (CT) and magnetic resonance imaging (MRI) of the brain were negative. Whole body CT and [18F] fluoro-2-deoxyglucose positron emission tomography confirmed the tumour invasion of tracheal and bronchial lymph nodes only. Neither a primary tumour nor other metastases could be identified. Serum screening for antineuronal antibodies was positive for anti-Yo (indirect immunofluorescence and western blot). In serum screening with immunofluorescence, antineuronal antibodies against the cytoplasm of Purkinje cells and brainstem neurones were found. The diluted sera from the patient and controls were applied to rat brain sections as described in detail previously [11]. Anti-Yo IgG antibodies were confirmed by immunoblot of Yo recombinant protein (Milenia Biotec GmbH, Bad Nauheim, Germany). Testing for anti-Hu, anti-Ri, anti-Ma, anti-CRMP5 and anti-Amphiphysin was negative. Tests used for the detection of antineuronal antibodies are approved for diagnostic use.

Routine blood examinations including other tumour markers (prostate specific antigen, cancer antigen 19–9; carcinoembryonic antigen, neurone specific enolase, cytokeratin-filament 19, beta2-microglobulin, and alpha-fetoprotein) were found within normal range. While gait ataxia worsened continuously, dysarthria and aphasia were fluctuating and seemed to change 'from day to day'. Receptive as well as expressive language modalities were alternately affected. Brain CT and MRI all applied with

contrast media were normal. Electroencephalogram was normal. Four weeks after onset of neurological symptoms chemotherapy with cisplatin/vinorelbine was initiated. Although, tracheal and bronchial lymph nodes were markedly reduced, and no primary tumour or further metastases could be found, the general condition and neurological symptoms deteriorated rapidly. The patient died 5 months after onset of neurological symptoms due to pneumonia.

Autopsy revealed a small adenocarcinoma of the lung and metastases in regional lymph nodes. No distant metastases were identified. Immunohistochemistry and confocal laser microscopy were carried out with essentially the same antibodies and protocols as described in detail previously [12–14]. In addition, well-characterized mouse monoclonal antibodies directed against CD56 (natural killer cells; clone 123C3; Monosan, Am Uden, the Netherlands) and Yo antigen (partial recombinant Yo antigen/CDR2 protein; clone 4F5; Abnova, Taipeh, Taiwan) were used. Tumour cells expressed Yo antigen (Figure 1A). The primary tumour and metastasis showed profound infiltration with CD3⁺/CD8⁺/Granyme B⁺/CD56⁺ cytotoxic T-lymphocytes (CTL) (Figure 1E). Staining for activated complement complex (C9neo) was negative. Brain vessels, cerebrum, brainstem and the cerebellum were macroscopically unremarkable. Within the central nervous system, Yo antigen – the immunogenic CDR-2 protein – was confined to Purkinje cells (Figure 1B, arrow), brainstem neurones and pyramidal neurones in perisylvian areas of both hemispheres (Figure 1C,D). In the cerebellar cortex widespread and nearly complete loss of Purkinje cells associated with Bergmann astrogliosis was found (data not shown) indicating a final 'burnt out' stage of chronic inflammation [4]. In a few areas clusters of activated microglia and CTL accumulated around some scattered preserved major histocompatibility complex (MHC) class I positive Purkinje cells (Figure 1F–H). Brainstem and cerebral cortex showed scattered parenchymal and perivascular infiltration of CTL (data not shown). No ischaemic changes were found. The cortical areas along the left and right perisylvian fissures showed profound activation of microglia and gliosis (Figure 1I–J), and expression of Yo antigen and MHC class I molecules in pyramidal cells (Figure 1C,D,K). In all other examined regions (frontal, temporal, parietal and occipital lobes) Yo antigen expression in neurones was absent or only very scattered. No activated complement complex (C9neo) was found (data not shown).

Efficient tumour defence requires both cellular and humoral mechanisms [1]. In anti-Yo PCD the humoral and cellular immune responses are strictly focused on specific Yo antigens. Yo antigen expressing tumour cells, Purkinje cells and brainstem neurones are killed by lysis or phagocytosis due to complement fixing anti-Yo antibodies or apoptosis due to Yo-specific CTL [2,4,5,15,16]. The selective expression of Yo antigens seen in anti-Yo PCD patients and the function of Yo antigens remains a matter of debate. The messenger RNA of one of their gene products, the CDR-2 protein (which contains the immunogenic epitope), can be detected in nearly all human tissues [15]. But CDR-2 protein expression was found in PCD patients restricted to Purkinje cells, brainstem neurones and tumour cells [2,3]. Posttranscriptional regulatory mechanisms might be responsible for the restricted protein expression [15]. The reason for this expanded expression of CDR-2 protein in neocortical neurones in our patient remains unclear. However, anti-Yo antibodies from patients' sera were found to bind on (scattered) neocortical neurones, and even to astrocytes and oligodendrocytes under conditions of activated protein synthesis in the adult rodent brain [17–19].

The expression of the immunogenic CDR-2 protein in neocortical areas was accompanied by inflammatory changes comparable with those seen in the cerebellum. Widespread loss of Purkinje cells and neocortical neurones with profound microglial activation and gliosis resemble, in both the cerebellar and the cerebral cortex, the final 'burnt out' stage of chronic inflammation [4]. Activation of microglia and infiltration of cytotoxic T cells were also found recently in the cerebral cortex of a woman suffering from anti-Yo PCD [20]. But it remains unclear whether humoral or specific cellular immune response or both might have caused the neuronal damage. Still ongoing inflammation was sparse and mild, and restricted to areas where neurones and Purkinje cells expressed CDR-2 protein and MHC class I molecules. However, the presence of CTL and lack of IgG or complement does not rule out specific humoral immune responses. The intact blood brain barrier is not an absolute barrier for immunoglobulins [21,22]. It is conceivable that the chemotherapy may have reduced the titres of anti-Yo IgG antibodies [23,24] to levels still detectable in serum but below the detection limit for immunohistochemistry.

In conclusion, our data provide three key observations: (i) further evidence of anti-Yo associated PCD in a male patient; (ii) expression of Yo antigen in neocortical

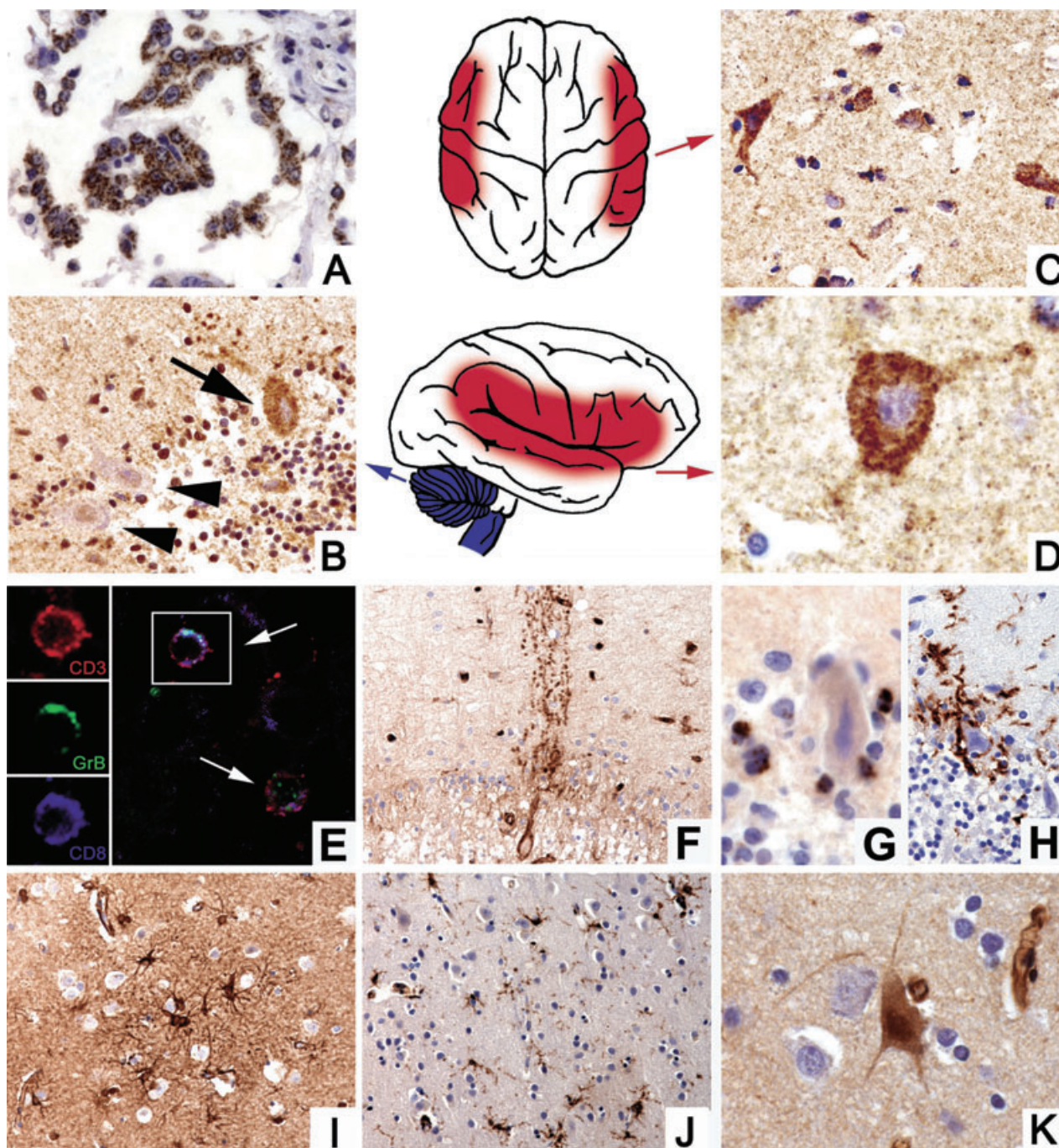


Figure 1. Scheme (middle panel): areas with Yo antigen expression in neurones and inflammatory changes, blue: cerebellum and medulla oblongata (detailed photograph, B); red: perisylvian regions on both hemispheres (detailed photographs, C,D). (A–D) detailed photographs (A–C, 400 \times and D, 600 \times). Immunohistochemistry with recombinant monoclonal Yo antibody. A, primary tumour. B, cerebellum: arrow, Yo antigen positive Purkinje cell and arrowheads, Yo antigen negative Purkinje cells. C,D, Yo positive neocortical neurones. (E) CD3⁺/CD8⁺/Granzyme B⁺ cytotoxic T cells (confocal image, 600 \times , arrows). (F) Major histocompatibility complex (MHC) class I expressing Purkinje cell (heavy chain of MHC class I, 200 \times). (G) Cytotoxic T-lymphocytes with polarized Granzyme B positive granules surround a Purkinje cell (Granzyme B, 600 \times). (H) focus of microglia in cerebellar cortex (HLA-DR, 400 \times). (I) profound gliosis (GFAP, 200 \times). (J) microglia cell activation (HLA-DR, 200 \times). (K) MHC class I expression on neurones (heavy chain of MHC class I, 600 \times).

neurones of both cerebral hemispheres; and (iii) degeneration of neocortical neurones in anti-Yo PCD with associated clinical symptoms (perceptive and receptive language disorder). Neither the expression of Yo antigen in neocortical neurones nor the associated immune attack nor the associated clinical symptoms have been reported before.

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References

- 1 Voltz R. Paraneoplastic neurological syndromes. an update on diagnosis, pathogenesis, and therapy. *Lancet Neurol* 2002; **1**: 294–305
- 2 Greenlee JE, Brashear HR. Antibodies to cerebellar Purkinje cells in patients with paraneoplastic cerebellar degeneration and ovarian carcinoma. *Ann Neurol* 1983; **14**: 609–13
- 3 Rodriguez M, Truh LI, O'Neill BP, Lennon VA. Autoimmune paraneoplastic cerebellar degeneration: ultrastructural localization of antibody-binding sites in Purkinje cells. *Neurology* 1988; **38**: 1380–6
- 4 Verschuuren J, Chuang L, Rosenblum MK, Lieberman F, Pryor A, Posner JB, Dalmau J. Inflammatory infiltrates and complete absence of Purkinje cells in anti-Yo-associated paraneoplastic cerebellar degeneration. *Acta Neuropathol (Berl)* 1996; **91**: 519–25
- 5 Albert ML, Darnell JC, Bender A, Francisco LM, Bhardwaj N, Darnell RB. Tumor-specific killer cells in paraneoplastic cerebellar degeneration. *Nat Med* 1998; **4**: 1321–4
- 6 Sutton IJ, Fursdon Davis CJ, Esiri MM, Hughes S, Amyes ER, Vincent A. Anti-Yo antibodies and cerebellar degeneration in a man with adenocarcinoma of the esophagus. *Ann Neurol* 2001; **49**: 253–7
- 7 Xia K, Saltzman JR, Carr-Locke DL. Anti-Yo antibody-mediated paraneoplastic cerebellar degeneration in a man with esophageal adenocarcinoma. *Medgenmed* 2003; **5**: 18
- 8 Meglic B, Graus F, Grad A. Anti-Yo-associated paraneoplastic cerebellar degeneration in a man with gastric adenocarcinoma. *J Neurol Sci* 2001; **185**: 135–8
- 9 Felician O, Renard JL, Vega F, Creange A, Chen QM, Bequet D, Delattre JY. Paraneoplastic cerebellar degeneration with anti-Yo antibody in a man. *Neurology* 1995; **45**: 1226–7
- 10 Matschke J, Kromminga A, Erbersdobler A, Lamszus K, Anders S, Kofuncu E. Paraneoplastic cerebellar degeneration and anti-Yo antibodies in a man with prostatic adenocarcinoma. *J Neurol Neurosurg Psychiatry* 2007; **78**: 775–7
- 11 Giometto B, Scaravilli T, Nicolao P, An SF, Groves M, Tavolato B, Beckett AA, Scaravilli F. Detection of paraneoplastic anti-neuronal autoantibodies on paraffin-embedded tissues. *Acta Neuropathol (Berl)* 1996; **92**: 435–40
- 12 Höftberger R, Aboul-Enein F, Brück W, Lucchinetti C, Rodriguez M, Schmidbauer M, Jellinger K, Lassmann H. Expression of major histocompatibility complex class I molecules on the different cell types in multiple sclerosis lesions. *Brain Pathol* 2004; **14**: 43–50
- 13 Aboul-Enein F, Rauschka H, Kornek B, Stadelmann C, Steffler A, Brück W, Lucchinetti C, Schmidbauer M, Jellinger K, Lassmann H. Preferential loss of myelin-associated glycoprotein reflects hypoxia-like white matter damage in stroke and inflammatory brain diseases. *J Neuropathol Exp Neurol* 2003; **62**: 25–33
- 14 Aboul-Enein F, Weiser P, Höftberger R, Lassmann H, Bradl M. Transient axonal injury in the absence of demyelination: a correlate of clinical disease in acute experimental autoimmune encephalomyelitis. *Acta Neuropathol (Berl)* 2006; **111**: 539–47
- 15 Corradi JP, Yang C, Darnell JC, Dalmau J, Darnell RB. A post-transcriptional regulatory mechanism restricts expression of the paraneoplastic cerebellar degeneration antigen cdr2 to immune privileged tissues. *J Neurosci* 1997; **17**: 1406–15
- 16 Tanaka K, Tanaka M, Tsukamoto T, Uehara T, Tsuji S. Anti-Yo antibody binding to 62-, 52-, or 58-kd protein recognizes a single molecule. *Neurology* 1996; **46**: 1777–8
- 17 Nardelli E, Curro-Dossi B, Rizzuto N, Graus F. Autoimmune paraneoplastic cerebellar degeneration: immunohistological localization of antibody-binding sites. *Clin Neurol Neurosurg* 1995; **97**: 89–94
- 18 Graus F, Illa I, Agusti M, Ribalta T, Cruz-Sanchez F, Juarez C. Effect of intraventricular injection of an anti-

¹equal contribution.

- Purkinje cell antibody (anti-Yo) in a guinea pig model. *J Neurol Sci* 1991; **106**: 82–7
- 19 Greenlee JE, Brashear HR, Herndon RM. Immunoperoxidase labelling of rat brain sections with sera from patients with paraneoplastic cerebellar degeneration and systemic neoplasia. *J Neuropathol Exp Neurol* 1988; **47**: 561–71
- 20 Giometto B, Marchiori GC, Nicolao P, Scaravilli T, Lion A, Bardin PG, Tavolato B. Sub-acute cerebellar degeneration with anti-Yo autoantibodies: immunohistochemical analysis of the immune reaction in the central nervous system. *Neuropathol Appl Neurobiol* 1997; **23**: 468–74
- 21 Reid DM, Perry VH, Andersson PB, Gordon S. Mitosis and apoptosis of microglia in vivo induced by an anti-CR3 antibody which crosses the blood–brain barrier. *Neuroscience* 1993; **56**: 529–33
- 22 Bechmann I, Galea I, Perry VH. What is the blood–brain barrier (not)? *Trends Immunol* 2007; **28**: 5–11
- 23 Galanis E, Frytak S, Rowland KM Jr, Sloan JA, Lennon VA. Neuronal autoantibody titres in the course of small-cell lung carcinoma and platinum associated neuropathy. *Cancer Immunol Immunother* 1999; **48**: 85–90
- 24 Zandvoort A, Lodewijk ME, Klok PA, Times W. Effects of multidose combination chemotherapy on the humoral immune system. *Clin Immunol* 2003; **107**: 20–9

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