Clinical analysis of anti-Ma2-associated encephalitis

Josep Dalmau,[1] Francesc Graus,[2] Alberto Villarejo,[1,*] Jerome B. Posner,[3] Deborah Blumenthal,[4] Brian Thiessen,[5] Albert Saiz,[2] Patricio Meneses[1] and Myrna R. Rosenfeld[1]

[1]Department of Neurology, University of Pennsylvania, Philadelphia, PA, USA, [2]Service of Neurology and Institut d'Investigacions Biomèdiques August Pí i Sunyer (IDIBAPS), Hospital Clínic, University of Barcelona, Spain, [3]Department of Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY, [4]Departments of Neurology, Neurosurgery, and Oncology, University of Utah School of Medicine/Huntsman Cancer Institute, UT, USA and [5]Division of Medical Oncology, British Columbia Cancer Agency, Vancouver, BC, Canada
*Present address: Service of Neurology, Hospital Universitario Doce de Octubre, University Complutense, Madrid, Spain

Correspondence to: Josep Dalmau, MD, PhD, Department of Neurology, 3 W. Gates, Division of Neuro-oncology, University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104, USA
E-mail: jdalmau@mail.med.upenn.edu

Summary

Increasing experience indicates that anti-Ma2-associated encephalitis differs from classical paraneoplastic limbic or brainstem encephalitis, and therefore may be unrecognized. To facilitate its diagnosis we report a comprehensive clinical analysis of 38 patients with anti-Ma2 encephalitis. Thirty-four (89%) patients presented with isolated or combined limbic, diencephalic or brainstem dysfunction, and four with other syndromes. Considering the clinical and MRI follow-up, 95% of the patients developed limbic, diencephalic or brainstem encephalopathy. Only 26% had classical limbic encephalitis. Excessive daytime sleepiness affected 32% of the patients, sometimes with narcolepsy-cataplexy and low CSF hypocretin. Additional hormonal or MRI abnormalities indicated diencephalic-hypothalamic involvement in 34% of the patients. Eye movement abnormalities were prominent in 92% of the patients with brainstem dysfunction, but those with additional limbic or diencephalic deficits were most affected; 60% of these patients had vertical gaze paresis that sometimes evolved to total external ophthalmoplegia. Three patients developed atypical parkinsonism, and two a severe hypokinetic syndrome with a tendency to eye closure and dramatic reduction of verbal output. Neurological symptoms preceded the tumour diagnosis in 62% of the patients. Brain MRI abnormalities were present in 74% of all patients and 89% of those with limbic or diencephalic dysfunction. Among the 34 patients with cancer, 53% had testicular germ-cell tumours. Two patients without evidence of cancer had testicular microcalcification and one cryptorchidism, risk factors for testicular germ-cell tumours. After neurological syndrome development, 17 of 33 patients received oncological treatment (nine also immunotherapy), 10 immunotherapy alone, and six no treatment. Overall, 33% of the patients had neurological improvement, three with complete recovery; 21% had long-term stabilization, and 46% deteriorated. Features significantly associated with improvement or stabilization included, male gender, age <45 years, testicular tumour with complete response to treatment, absence of anti-Ma1 antibodies and limited CNS involvement. Immunosuppression was not found to be associated with improvement but was clearly effective in some patients. Fifteen patients (10 women, five men) had additional antibodies to Ma1. These patients were more likely to have tumours other than testicular cancer and to develop ataxia, and had a worse prognosis than patients with only anti-Ma2 antibodies (two women, 21 men); 67% of deceased patients had anti-Ma1 antibodies. Anti-Ma2 encephalitis (with or without anti-Ma1 antibodies) should be suspected in patients with limbic, diencephalic or brainstem dysfunction, MRI abnormalities in these regions, and inflammatory changes in the CSF. In young male patients, the primary tumour is usually in the testis, in other patients the leading neoplasm is lung cancer.

Introduction

Paraneoplastic limbic and brainstem encephalitis are two syndromes with well described clinicopathological features that are likely immune-mediated (Dropcho, 2004). Paraneoplastic limbic encephalitis typically causes short-term memory loss, seizures, irritability, depression and cognitive decline that may evolve to dementia (Hierons *et al.*, 1978; Posner, 1995). Pathological and neuroradiological studies localize the major abnormalities in the medial temporal lobes, hippocampi and amygdala (Gultekin *et al.*, 2000). Paraneoplastic brainstem encephalitis causes a variety of deficits that result from an inflammatory degeneration of the lower brainstem, typically involving the inferior olives and dorsal nuclei of the medulla (Henson and Urich, 1982). The presentation of these syndromes regularly precedes the diagnosis of the cancer, complicating their recognition as a paraneoplastic neurological disorder (PND) (Posner, 1995). In previous studies we described the molecular characteristics of the Ma onconeuronal proteins and indicated that patients with antibodies to these proteins usually develop limbic or brainstem encephalitis (Rosenfeld *et al.*, 2001). Increasing experience has made it apparent that the clinical features of these patients, along with the MRI and pathological findings, define a disorder that usually differs from classical paraneoplastic limbic or brainstem encephalitis. The resulting atypical syndrome may go unrecognized for several months. With the goal of improving the recognition of this disorder, the current study focuses in the comprehensive clinical description of 38 patients, their long-term follow-up, and the factors associated with neurological improvement and stabilization.

Patients and methods

Patients, sera and CSF

We reviewed the clinical data of all patients with anti-Ma1 and anti-Ma2 antibodies diagnosed in two reference laboratories for PND. Clinical and pathological information was provided by the referring physicians or obtained by the authors that personally examined 12 patients. All patients had brain MRI or computed tomography (CT), CSF studies or clinical follow-up that ruled out other neurological complications of cancer. Neurological symptoms were coded into the following syndromes: limbic encephalitis (Gultekin *et al.*, 2000), diencephalic encephalitis, brainstem encephalitis and other. Criteria for limbic encephalitis included short-term memory loss, seizures, confusion, hallucinations, mood disorder or personality change; the demonstration of MRI abnormalities in the limbic system was required if the predominant deficit was other than short-term memory loss. Criteria for diencephalic encephalitis included excessive daytime sleepiness (EDS), narcolepsy-cataplexy, decrease of CSF hypocretin, hyperthermia, hypothalamic-pituitary endocrine dysfunction, gain of weight or sexual dysfunction; the demonstration of MRI abnormalities in the diencephalon was required if the predominant symptom was EDS, hyperthermia, weight gain or sexual dysfunction. Brainstem encephalitis was considered when patients developed cranial neuropathy, nuclear or supranuclear ophthalmoparesis, parkinsonism, dysarthria or dysphagia; the demonstration of MRI abnormalities in the brainstem was required if the patient had predominant parkinsonism, dysarthria, dysphagia, and asymmetric or mild ataxia.

The response to treatment was assessed as described previously (Graus *et al.*, 2001). The neurological disorder was considered improved if there was a decrease in the modified Rankin score of at least 1 point at the last visit. Analysis for anti-Ma1 and anti-Ma2 antibodies was confirmed using immunoblot of recombinant Ma1 and Ma2 proteins as reported in a single laboratory (J.D.) (Dalmau *et al.*, 1999; Rosenfeld *et al.*, 2001). Thirty-eight patients were included; the detailed description of 10 patients (Bennett *et al.*, 1999; Dalmau *et al.*, 1999; Sutton *et al.*, 2000; Barnett *et al.*, 2001; Wong *et al.*, 2001; Landolfi and Nadkarni, 2003) and the antibody findings of another 19 have been reported previously (Voltz *et al.*, 1999; Rosenfeld *et al.*, 2001). The comprehensive clinical description and long-term follow-up of 28 patients have not been reported. Fisher's exact test was used for analysis of prognostic factors. This study was approved by The Institutional Review Board of The University of Pennsylvania.

Results

Twenty-six patients were male (68%) with a median age of 34 years (range 22–70 years), and 12 were female (32%) with a median age of 64 years (range 53–82 years). All patients' sera and CSF (available from 13 patients) had anti-Ma2 antibodies. Fifteen patients had both anti-Ma1 and anti-Ma2 antibodies. The median immunoblot serum titre of anti-Ma2 antibodies was 1 : 128 000 (range 1 : 4000 to 1 : 1 024 000), and anti-Ma1 antibodies 1 : 16 000 (range 1 : 1000 to 1 : 128 000). Using a previously reported method intrathecal synthesis of anti-Ma2 antibodies was demonstrated in eight out of 10 patients (Voltz *et al.*, 1999); intrathecal synthesis of anti-Ma1 antibodies was not determined. None of the patients had other onconeuronal antibodies.

Relationship of neurological symptoms to tumour diagnosis

Neurological symptoms developed before the tumour diagnosis in 21 (62%) patients (median 6 months, range 1–36 months), and after the tumour diagnosis in 13 (38%) patients (median 9 months, range 2 months to 7 years). In two of them

the PND preceded the recurrence of a cancer that had been diagnosed 9 months and 7 years earlier. In four patients no tumour has been identified.

Neurological syndromes

The main syndromes of the 38 patients are shown in Table 1. Thirty-four patients developed symptoms of limbic, diencephalic or brainstem dysfunction, usually combining several areas of involvement (Fig. 1), and four patients developed other syndromes.

Table 1 *Neurological syndromes in 38 patients with immunity to Ma proteins*

Predominant syndrome*	No. of patients	Accompanying deficits[†]
Limbic	7	None
Diencephalic	1	None
Limbic and diencephalic	1	None
Brainstem with limbic and/or diencephalic	20	Ataxia in 9 patients
Brainstem	5	Ataxia in 2 patients; hypothalamic, thalamic and periaqueductal MRI findings in 1 patient
Cerebellar ataxia	2	None
Myelopathy	1	Anxiety, depression, memory problem, paranoia, hallucinations
Myelopathy, radiculo-plexopathy	1	Brainstem

*Syndrome responsible for most or all the functional disability.
[†]Syndromes defined by less severe symptoms, neurological signs or asymptomatic MRI abnormalities.
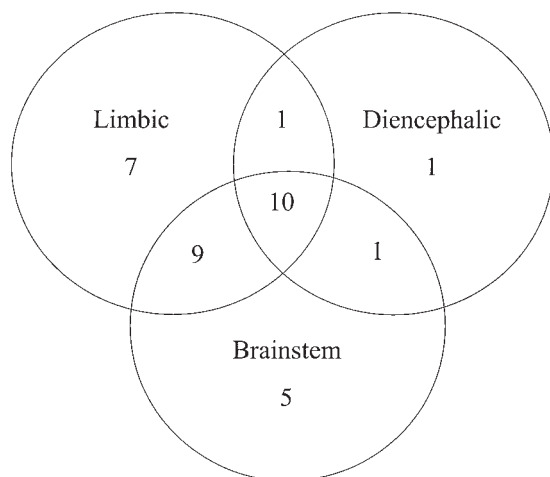

Fig. 1 Distribution of predominant syndromes in 34 patients with anti-Ma2 encephalitis. Twenty-one patients developed symptoms of multifocal involvement of the limbic system, diencephalon or brainstem. Thirteen patients developed unifocal involvement of these areas.

The median time between the first neurological symptom and stabilization or death was 6 months (range 5 days to 16 months). The neurological disease was monophasic in 35 patients. Three patients had neurological relapse, two associated with systemic tumour recurrence, and one while decreasing the dose of immunosuppressants.

Limbic-diencephalic-brainstem syndromes
Presenting symptoms
The initial symptoms of these patients are shown in Table 2. Eleven of the 34 patients presented with typical symptoms of limbic encephalopathy, seven as an isolated syndrome and four with accompanying hypothalamic deficits. One patient with recent history of a germ-cell tumour developed psychomotor seizures as the only symptom of limbic encephalitis, supported by the MRI findings and the detection of anti-Ma2 antibodies in serum and CSF (Fig. 2A). One patient presented with isolated hypothalamic dysfunction. Five patients presented with a brainstem syndrome. Sixteen patients developed a complex mixture of brainstem, limbic or diencephalic dysfunction.

Clinical features of the established syndrome
The fully developed syndrome of these 34 patients included limbic encephalopathy in 27 patients, diencephalic dysfunction in 13 and brainstem encephalopathy in 25. Most patients had a combination of several of these features (Fig. 1). Only 13 patients had symptoms of involvement of one single area: seven classical limbic encephalopathy, one diencephalic dysfunction and five brainstem encephalopathy.

Of 27 patients with limbic encephalopathy, 24 had severe short-term memory deficits; two had a confusional state and decline of cognitive function that did not allow examination of memory, but the MRI showed involvement of the limbic system, and one patient had normal memory. The latter had isolated psychomotor seizures and has not developed new symptoms during a 7 month follow-up. Overall, 12 patients had clinical evidence of seizures (seven partial complex seizures; five partial complex seizures and generalized tonic-clonic seizures). The EEG studies obtained in 10 patients all demonstrated unilateral or bilateral epileptic foci mainly involving the temporal lobes. One patient without clinical evidence of seizures had periodic lateral epileptiform discharges (PLEDS) that predominated in the right frontotemporal lobe.

Thirteen patients developed diencephalic dysfunction, 12 with EDS and two of them with cataplexy and hypnagogic hallucinations. In five patients with EDS the CSF hypocretin level was very low or undetectable; in two patients without EDS the CSF hypocretin levels were normal (Overeem *et al.*, 2004). Three patients developed unexplained weight gain (20, 23 and 136 kg), three hyperthermia and four various endocrine abnormalities [one decreased cortisol, one diabetes insipidus and hypothyroidism, one syndrome of inappropriate secretion of antidiuretic hormone (SIADH), and one panhypopituitarism with diabetes insipidus].

Table 2 *Presenting symptoms of 34 patients with limbic-diencephalic-brainstem syndromes*

Syndrome (no. of patients)	Clinical features
Limbic (7)	All with typical features of limbic encephalitis
Limbic-hypothalamic (4)	3 EDS, 1 decrease of libido and weight gain
Psychomotor seizures (1)	MRI and CSF findings supportive of limbic encephalitis
Hypothalamic (1)	EDS, diabetes insipidus, confusion, disorientation, low serum FSH, LH, ACTH, TSH
Brainstem (5)	2 diplopia and unsteadiness, 1 oscillopsia, 2 dysphagia, 2 dysarthria
Brainstem-limbic-diencephalic (16)	Visual or eye movement deficits: 8 diplopia, 1 opsoclonus, 2 difficulty opening the eyes
	Psychiatric symptoms: 2 'nervous breakdown', 1 loss of self-confidence, 1 panic attacks
	Seizures: 4 complex partial seizures; 1 complex partial and generalized seizures
	Hypothalamic symptoms: 2 EDS, 1 hyperthermia, 1 loss of libido, diabetes insipidus and hypothyroidism
	Other: 2 lethargy, 5 unsteadiness or mild gait ataxia

ACTH = adrenocorticotropic hormone; FSH = follicle-stimulating hormone; LH = luteinizing hormone; TSH = thyroid-stimulating hormone.
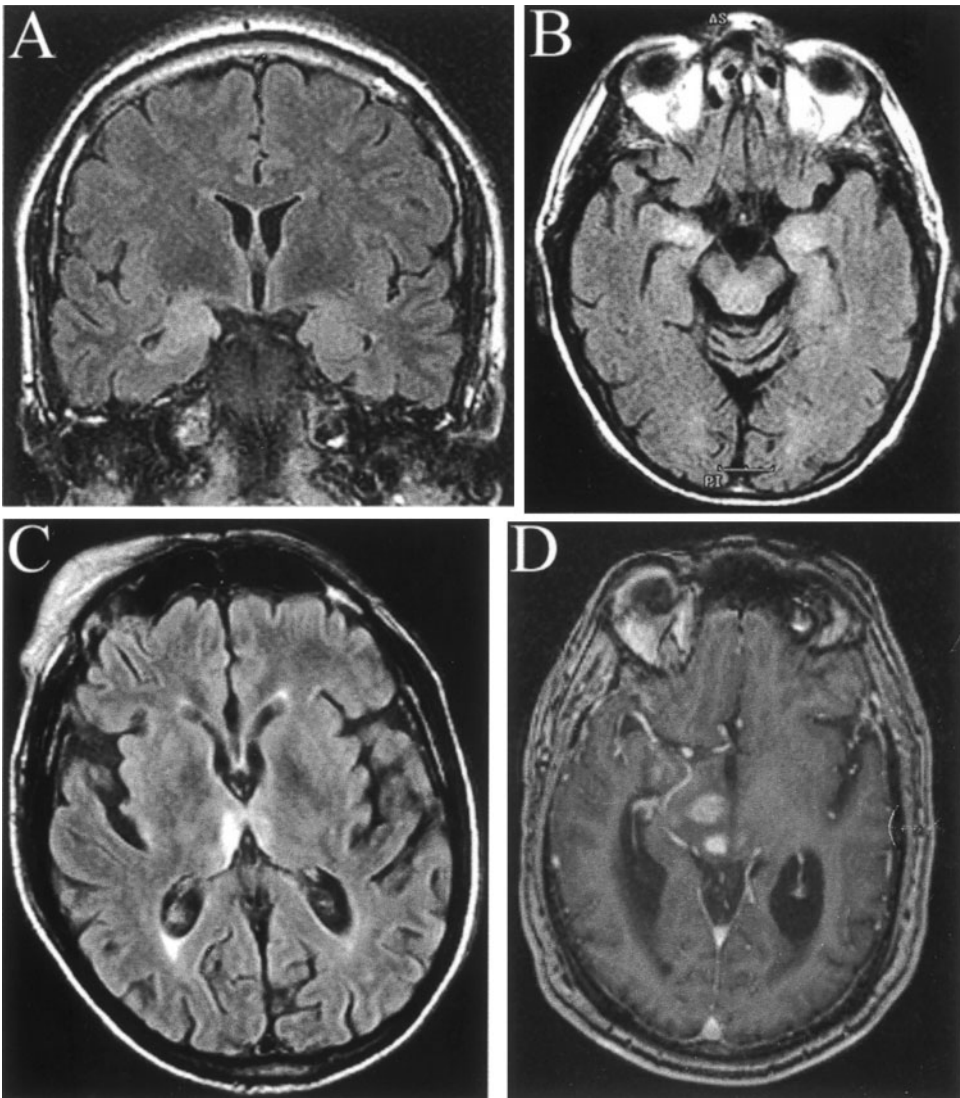

Fig. 2 MRIs of patients with anti-Ma2 encephalitis. (**A**) MRI FLAIR sequence of a patient with testicular germ-cell tumour and isolated temporal lobe seizures. Note the presence of asymmetric abnormalities in the temporal lobes; the CSF was positive for oligoclonal bands and anti-Ma2 antibodies. (**B** and **C**) MRI FLAIR sequences of a patient with severe hypokinetic syndrome, non-paretic eye closure and reduction of verbal output, showing abnormalities in the mesial temporal lobes and dorsal mesencephalon (**B**), and medial thalami (**C**). (**D**) T1-weighted MRI of a patient with non-SCLC and anti-Ma2 encephalitis, showing nodular areas of contrast enhancement in the right temporal lobe, thalamic, subthalamic and collicular regions. Biopsy of one of the lesions demonstrated perivascular and interstitial infiltrates of mononuclear cells and plasma cells.

Twenty-five patients developed brainstem encephalopathy, 20 combined with limbic or diencephalic symptoms, and five as an isolated syndrome. Twenty-three of these 25 (92%) patients had eye movement abnormalities. Vertical gaze paresis (VGP) that evolved to severe or total paralysis occurred in 12 out of 20 (60%) patients with combined brainstem, diencephalic or limbic symptoms, and one out of five (20%) patients with pure brainstem dysfunction. By the time that the VGP manifested, the oculocephalic manoeuvre or Bell's phenomenon could partially overcome the deficit. Three of these patients eventually developed complete horizontal paralysis and VGP, and another two patients initially developed total VGP followed by complete horizontal paralysis of one eye and partial limitation of the other. Most patients developed upward saccadic deficits first, but two patients complained of initial difficulty looking down (noted while eating), and the examination demonstrated bilateral upward deviation of the eyes. Eyelid ptosis occurred in four patients; in three it was bilateral and in one unilateral. Two patients that could open their eyes kept them closed as part of a hypokinetic syndrome described below in more detail. Other neuro-ophthalmologic findings included: opsoclonus in three patients, ocular flutter in one, skew deviation in three, oculogyric crisis in one, saccadic gaze pursuit in four, paresis of internal rectus in three and superior oblique in one, Horner's sign in one, and nystagmus in six (four horizontal, one downbeating, one horizontal-rotatory). Some of these findings occurred in patients that also developed VGP. Non-eye movement abnormalities included, mild to moderate dysarthria in 10 patients, moderate to severe dysphagia in eight (three of them requiring percutaneous oesophagogastrostomy), bilateral facial weakness in three (two with myokymia), unilateral facial weakness in one, dystonic closure of the jaw in two, weakness of the masseters in one, sensorineural hearing loss in two (one bilateral) and facial hypoesthesia in one.

Less common clinical features

Three patients with brainstem dysfunction developed features of atypical parkinsonism. Two of them developed VGP, decreased blinking, facial masking, rigidity, tremor and hyperreflexia; one had an upgoing toe and the other dystonic postures. The third patient developed VGP, hypophonia, hypomimia and a paraparetic-ataxic gait.

Two other patients developed a severe hypokinetic syndrome with reduction of verbal output, and tendency to continuous eye closure without evidence of ptosis. One presented with lethargy, loss of libido, diabetes insipidus and hypothyroidism that evolved to a clinical picture of complete immobility and severe spasticity and rigidity. The patient stopped speaking and eating, had prominent EDS, but was able to respond 'thumbs up or down' with good accuracy when answering autobiographic questions. The MRI showed bilateral abnormalities involving the hippocampi, amygdala, midbrain (substantia nigra), internal capsule and globus pallidi. The other patient presented with loss of self-confidence, an unexplained sense of fear and diplopia, and also developed a

disorder characterized by hypokinesis. This patient had EDS (with low CSF hypocretin levels) and VGP, and kept his eyes closed all the time. Attempts by the examiner to open his eyes elicited a reflex blepharospasm. When prompted to speak, the patient barely moved the lips and spoke with inaudible hypophonia. Despite the appearance of being continuously sleeping, he was able to promptly follow commands, such as raising his arms or standing from a chair. He had a short-step gait resembling that of a parkinsonian patient. The initial MRI showed bilateral abnormalities involving the hippocampi, dorsal mesencephalon and colliculi (Fig. 2B); follow-up MRI revealed additional abnormalities in the medial thalami (Fig. 2C). The patient died in a nursing home as a result of progressive neurological deterioration 12 months after developing the neurological syndrome; no autopsy was obtained.

Eleven patients developed ataxia. Only one had predominant cerebellar dysfunction at symptom presentation. In eight patients the cerebellar deficits were mild to moderate; three of them had asymmetric involvement of the extremities and the other five truncal and gait ataxia. The remaining three patients developed severe limb and truncal ataxia.

Neuroimaging studies

At symptom presentation 33 of the 34 patients had brain MRI and seven also had CT studies. No neuroimaging information is available from one patient. Two of the seven patients with brain CT scans had abnormal findings; one had a hypodense abnormality in the right temporal lobe that enhanced with contrast, and the other had asymmetric bitemporal and right hypothalamic hypodensities that enhanced with contrast. Twenty-three of the 33 patients with initial brain MRI had abnormal findings; five had follow-up studies and all showed new abnormalities. Three of the 11 patients with normal initial MRI developed abnormalities in subsequent studies; no follow-up MRI was obtained for the other eight patients.

Among the 26 patients with MRI abnormalities, 25 had limbic or diencephalic symptoms (with or without brainstem symptoms), and one a predominant brainstem syndrome. When considering the medial temporal lobes as part of a single area (limbic system), 10 patients had one area and 16 more than one area involved. Overall, 18 patients had bilateral ($n = 14$) or unilateral ($n = 4$) medial temporal lobe abnormalities, two had abnormalities in the amygdala of the hippocampi, eight in the hypothalamus, four in the thalamus (two bilateral), three in the basal ganglia, eight in the midbrain (three superior colliculi, two periaqueductal region, one superior colliculi and periaqueductal region, one dorsal midbrain, one substantia nigra), two in the pons, one in the anterior medulla, one in the superior and middle cerebellar peduncles, and one had cerebellar atrophy. Except for the latter, the abnormalities of all patients were clearly demonstrated in T2-weighted and fluid-attenuated inversion recovery (FLAIR) sequences (Figs 2A–C and 3A). The T1-weighted sequences without contrast administration were usually normal or mildly hypointense; in 10 patients one
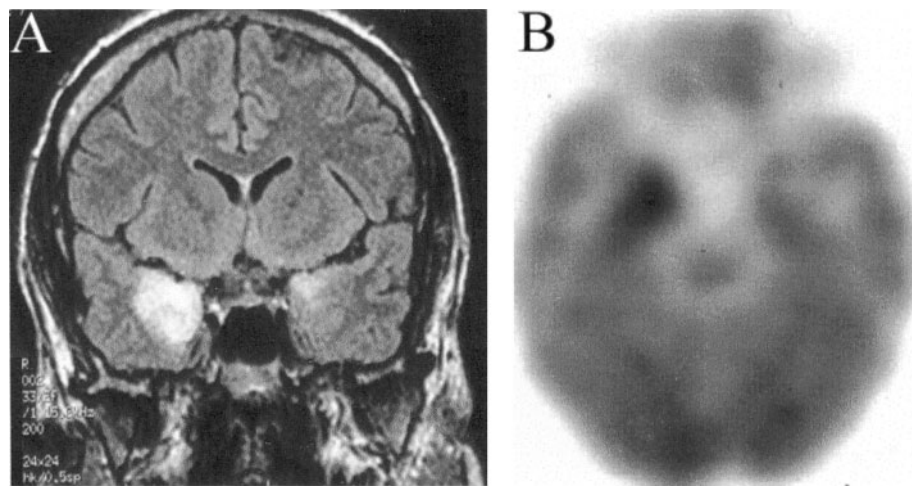
Fig. 3 MRI and FDG-PET of a patient with asymmetric limbic encephalitis. MRI FLAIR sequence (**A**) and FDG-PET (**B**) of a patient with limbic encephalitis without seizures. Note the asymmetric MRI abnormalities in the temporal lobes, and abnormal hypermetabolism in the right temporal lobe.

or more abnormalities showed enhancement after contrast administration (Fig. 2D).

Besides the brain MRI one patient with limbic encephalitis had MR spectroscopy and [^{18}F]fluoro-2-deoxyglucose (FDG)-PET. The MR spectra showed increased choline and acetate and decreased *N*-acetyl-aspartate in the area maximally involved in the MRI that correlated with the presence of hypermetabolism in the PET study (Fig. 3A and B). Another patient with limbic encephalitis and MRI showing bilateral medial temporal lobe abnormalities also had FDG-PET scan of the brain that showed hypermetabolism in one of the temporal lobes (data not shown).

Other syndromes

Four patients developed other syndromes. Two patients had a predominant cerebellar ataxia that remained stable for several years, without MRI abnormalities other than cerebellar atrophy. The other two patients developed a myelopathy (and one possible involvement of nerve roots and brachial plexus), with minimal signs of involvement of the limbic system and brainstem at the terminal stage of their disease. The initial course of the disease of one of the patients was reported previously (Rosenfeld *et al.*, 2001). She was a 72-year-old woman with a pulling-jumping sensation in the legs and bladder urgency that preceded by 8 years the diagnosis of a follicular lymphoma. After the lymphoma was diagnosed, her symptoms worsened and she developed pain 'like a toothache' in the feet, shaky handwriting, anxiety, depression, decreased hearing in the right ear and subtle memory problems. CSF studies and MRI of brain and spine were unrevealing. The clinical follow-up continued until her death in 2002 (4 years after the diagnosis of lymphoma). Three months before her death she developed paranoia and visual hallucinations requiring hospital admission and treatment with mirtazapine and olanzapine; no autopsy was obtained.

The other patient was a 58-year-old man who, 14 months after the diagnosis of adenocarcinoma of the lung, developed subacute shoulder pain, proximal weakness of the upper extremities, absent biceps and brachioradialis reflexes, muscle atrophy, fasciculations, and segmental hypoesthesia involving C4-C8 dermatomes. The CSF showed normal glucose, mild pleocytosis, elevated protein concentration, one oligoclonal band and cytology negative for malignant cells. The brain and spine MRI were normal. Eventually the patient developed nystagmus. He died 5 months after developing the neurological deficits; no autopsy was obtained.

CSF and neuropathological studies

CSF analysis was abnormal in 25 out of 32 patients. Eleven patients had increased protein concentration and pleocytosis, seven had increased protein concentration with normal cell count, five had pleocytosis with normal protein concentration, and two had normal cell count and protein concentration but with positive oligoclonal bands. Overall the pleocytosis ranged from five to 113 white blood cells (WBC)/mm^3 (median 20 WBC/mm^3) with predominant lymphocytes, and the total protein ranged from 47 to 747 mg/dl (median 71 mg/dl). The glucose concentration was always normal, and the cytology studies were negative for malignant cells. Oligoclonal bands were detected in six of eight patients.

Pathological studies were obtained in 12 patients; seven had biopsy (four in the temporal lobe, two in the hypothalamus and one in the thalamus) and five autopsy studies. In 11 patients the microscopic studies showed perivascular lymphocytic cuffing, and interstitial infiltrates of lymphocytes with variable gliosis and neuronal degeneration. The inflammatory infiltrates were mainly composed of T-cells, with a smaller number of B-cells, macrophages and microglial activation (Fig. 4A–C). Three patients had numerous plasma cells in the lymphocytic infiltrates. In one patient the prominent lymphocytic infiltrates
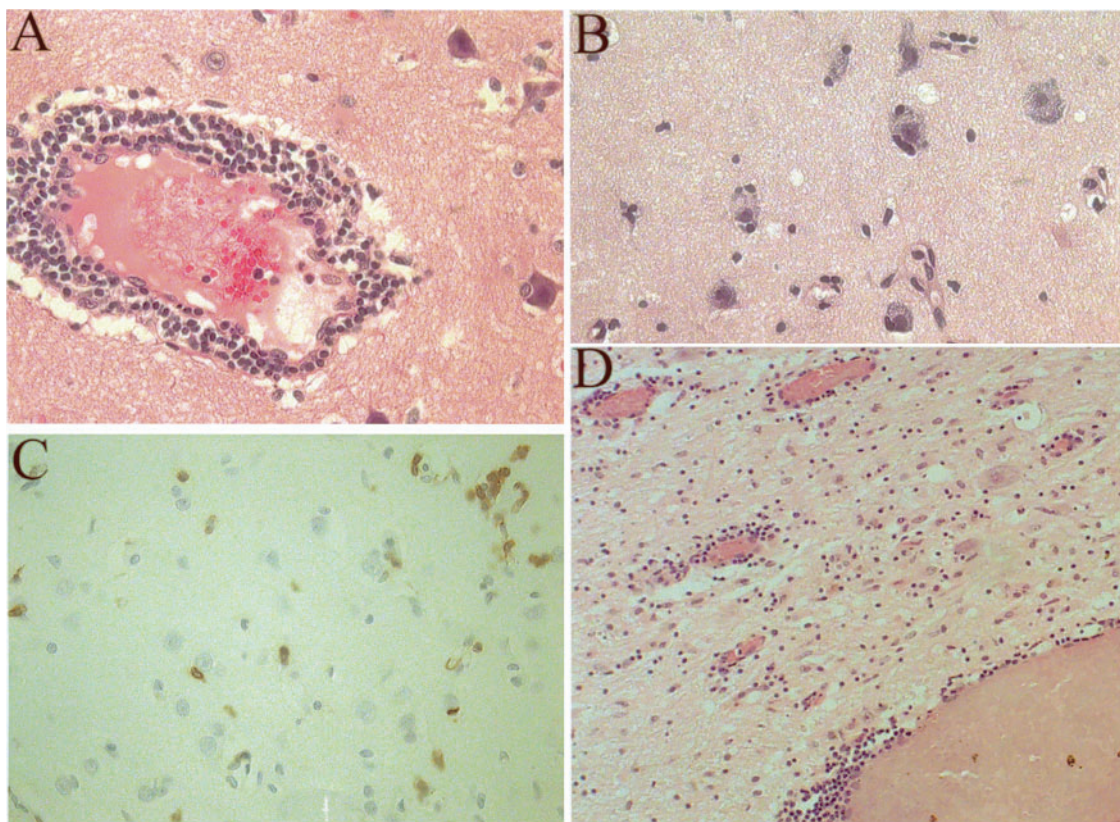
Fig. 4 Neuropathological findings in Ma2 encephalitis. Temporal lobe biopsy of a patient with limbic-diencephalic encephalitis (**A–C**), showing perivascular lymphocytic cuffing (**A**), T-lymphocytes in close contact with neurons (**B**) and immunophenotyping of these lymphocytes as CD8 $^+$ T cells (**C**). (**D**) Autopsy of a patient with brainstem encephalitis; the section demonstrates perivascular cuffing and extensive interstitial infiltrates of lymphocytes. **A** and **B**, haematoxylin–eosin, ×200; **C**, counterstained with haematoxylin, ×200; **D**, haematoxylin–eosin, ×80.

initially suggested a malignant lymphoproliferative disorder. The only patient without inflammatory infiltrates had a 7-year history of breast cancer, paraneoplastic cerebellar degeneration and cerebellar atrophy in the MRI. She died as a result of rapid tumour recurrence and sepsis; the autopsy showed extensive loss of Purkinje cells.

The autopsy of the other four patients showed a correlation between the clinical MRI findings and the degree of pathological involvement. The areas with major pathological abnormalities were always the most symptomatic, but all four patients had abnormal findings in areas that were clinically asymptomatic. In a patient whose symptoms appeared restricted to limbic and hypothalamic dysfunction, with episodes of cataplexy, the autopsy showed severe involvement of the hippocampus and entorhinal cortex, and mild chronic inflammation in numerous areas including frontal and parietal cortex, midbrain, susbtantia nigra, pontine nuclei, dorsal grey matter of the medulla, olivary nuclei and occasional dropout of Purkinje cells with Bergmann's gliosis. The spinal cord was spared, but the dorsal root ganglia showed occasional nodules of Nageotte and several small aggregates of reactive lymphocytes. The second patient had opsoclonus and personality change that evolved to brainstem encephalopathy, limbic dementia and cerebellar ataxia. The autopsy showed encephalitis affecting the cerebellar roof nuclei, periaqueductal grey matter, tectum, pretectum, superior and inferior colliculi, substantia nigra, medial geniculate body, pulvinar, and interstitial nucleus of Cajal (Wong *et al.*, 2001).

The third and fourth patients have been reported previously by our group (Dalmau *et al.*, 1999; Barnett *et al.*, 2001); both had in common the development of severe brainstem dysfunction, external ophthalmoplegia, dysphagia, abnormal MRI findings in the pons (and basal ganglia and superior cerebellar peduncles in one case), and extensive pathological abnormalities in the temporal lobes, diencephalon and throughout the brainstem (Fig. 4D). Focal loss of Purkinje cells was noted in both patients; one had mild involvement of the substantia nigra indicated by the presence of phagocytes with engulfed neuromelanin granules.

Oncological features

Pathological confirmation of cancer was obtained in 33 patients and radiological evidence of FDG-PET-positive mediastinal adenopathies in one. Eighteen patients had testicular cancer, seven lung cancer and nine other cancers (Table 3).

Table 3 *Associated tumours*

Associated tumours	n
Testicular tumours	18
Non-seminomatous*	12
Seminoma	4
Carcinoma *in situ* (intratubular germ-cell neoplasia)	2
Lung cancer[†]	7
Other[‡]	9
No tumour	4

*Non-seminomatous testicular tumours: four embryonal carcinoma, two mixed embryonal carcinoma and mature teratoma, one intratubular germ-cell tumour and teratoma, one mixed embryonal carcinoma, teratoma and endodermal sinus tumour, one mixed embryonal carcinoma and endodermal sinus tumour, one mixed embryonal carcinoma, mature teratoma and seminoma, one inmature teratoma, and one mixed seminoma and mature teratoma. [†]Lung cancer: three adenocarcinoma, two large-cell carcinoma, one pleural metastasis of adenocarcinoma and one positive PET mediastinal adenopathies without pathology. [‡]Other: two breast, one parotid gland, one ovary, one colon, one kidney, one lymphoma, one metastatic extragonadal choriocarcinoma and one metastasis of unknown primary.

None of the patients had more than one primary tumour; one patient with colon cancer had Castleman's disease involving the thymus. At the time of tumour diagnosis, 24 patients (15 with testicular tumour, nine with other tumours) had limited stage disease and 10 patients (three with testicular tumours and seven with other tumours) had metastasis.

In 12 patients the detection of anti-Ma2 antibodies prompted the search for the tumour, which in three cases was particularly difficult to demonstrate. Two of these patients underwent orchiectomy for a presumed testicular tumour suggested by the presence of serum anti-Ma2 antibodies and the development of ultrasound abnormalities in follow-up studies; both had carcinoma *in situ* (or intratubular germ-cell neoplasia) surrounded by inflammatory infiltrates (Fig. 5A and B). The third patient had a positive chest FDG-PET, with two negative biopsies of the suspicious lung lesion; the lung tumour was demonstrated at autopsy. Twelve tumours were examined by immunohistochemistry and all showed expression of Ma proteins (Fig. 5C–F).

In four patients the presence of a tumour could not be demonstrated. One patient has been lost to follow-up. The other three patients underwent extensive evaluation, including chest and abdomen CT and body FDG-PET. The first patient was a 23-year-old man with subacute development of limbic and hypothalamic dysfunction and ultrasound studies showing microcalcification in the right testis; he died 6 months after symptom presentation, and no tumour was found at autopsy. The second patient is a 36-year-old man with limbic encephalitis, whose testicular ultrasound showed a right-sided lesion suggesting a germ-cell tumour; he had a history of unilateral cryptorchidism with spontaneous descent into the scrotum at the age of 10 years. An orchiectomy demonstrated replacement of most of the testicular parenchyma by a firm, white zone that microscopically consisted of dense collagen; the remaining tissue showed atrophy of the seminiferous epithelium and tubular microcalcifications. The patient has been followed for 18 months without evidence of tumour and stable neurological deficits. The third patient had a predominant hypokinetic-parkinsonian syndrome; the body FDG-PET study showed two areas of hypermetabolism in the colon and prostate. The colonoscopy was negative and the serum prostate-specific antigen (PSA) normal; no prostate tissue or further studies were obtained and the patient died without autopsy being performed.

Treatment and outcome

Clinical outcome was obtained in 33 patients. After developing the neurological syndrome, 17 patients received oncological treatment and nine of them also received immunotherapy [three steroids, one steroids and intravenous immunoglobulin (IVIg), one steroids, plasma exchange and cyclophosphamide, one steroids and plasma exchange, one IVIg, one IVIg and plasma exchange, and one IVIg and IgG absorption with a protein A column]: eight patients improved, six stabilized and three deteriorated. Ten patients only received immunotherapy (four steroids and IVIg, three steroids and plasma exchange, two steroids, and one plasma exchange, IVIg and steroids); six of them had a previous history of tumour treatment (median 7 months, range 3–20 months) but developed the neurological syndrome while the tumour was in remission, one had extensive tumour recurrence that was not treated, and three had no tumour diagnosis: three patients improved, one stabilized and six deteriorated. Six patients did not receive oncological and/or immunological treatment and all deteriorated.

Seven of 12 patients with seizures required more than one anti-epileptic medication. For most of these patients the seizures remained an important problem; one patient had vagal nerve stimulation and another temporal lobectomy in an attempt to treat the seizures. The three patients with parkinsonian features were treated with levodopa; two had partial improvement and one did not improve. In one of the patients, attempts to discontinue the levodopa resulted in worsening tremor, and he was eventually treated with bromocriptine. In addition to bromocriptine the patient also received immunosuppression (steroids, plasma exchange) and at follow-up 3 years later he showed significant improvement; he was able to drive and use a bicycle, but remained severely dysarthric and mildly dystonic. One of the two patients with the severe hypokinetic syndrome received multiple treatments including bromocriptine, diazepam, dantrolene, dexamphetamine, methylprednisolone and IVIg without significant improvement.

Overall, 11 patients had objective neurological improvement (median follow-up 2.5 years, range 9 months to 6 years) with a median post-treatment Rankin score of 2 (Table 4), and seven had neurological stabilization (median follow-up 3.5 years, range 1–9 years) with a median Rankin score of 4. Improvement or stabilization occurred more frequently in
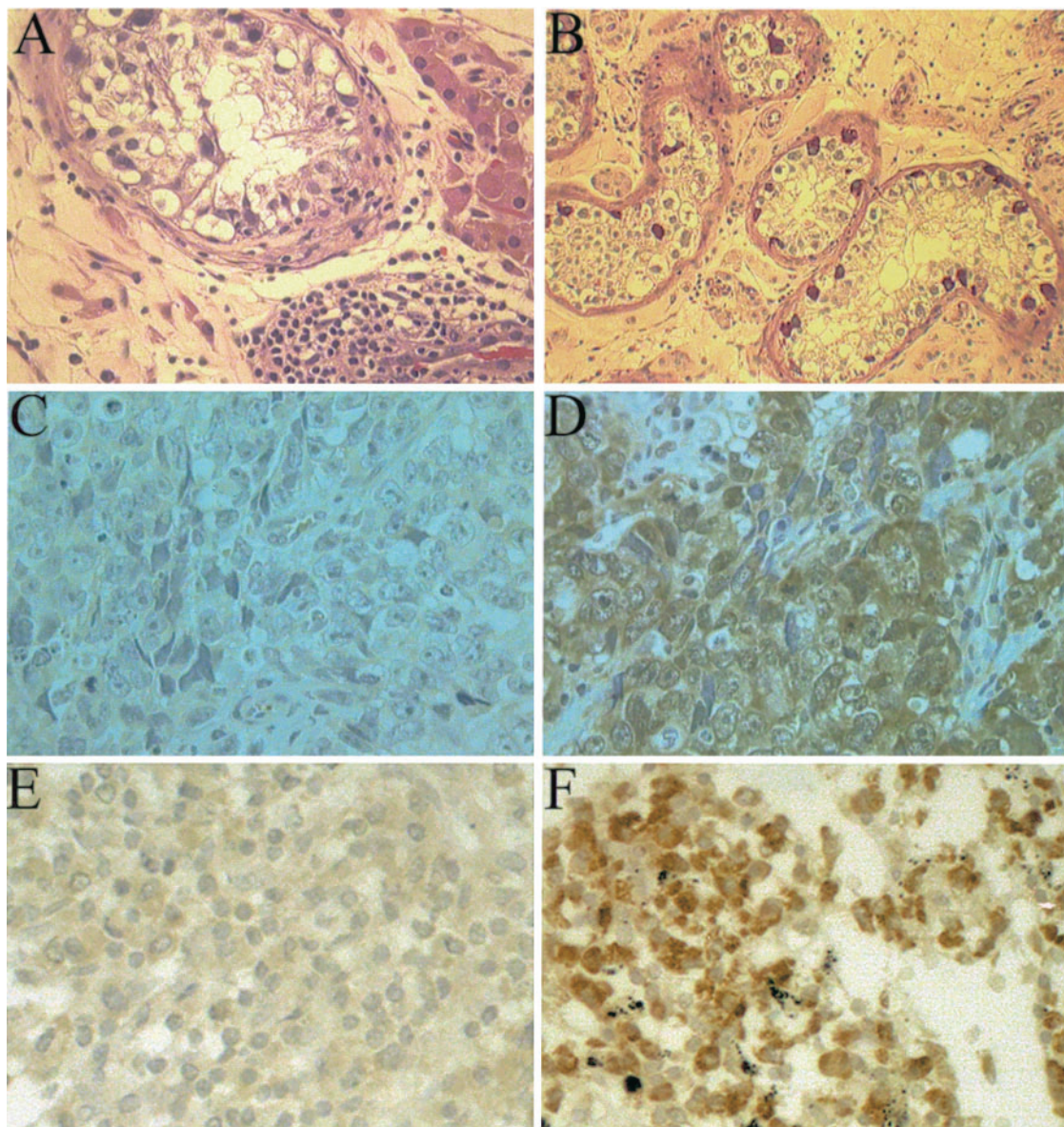
Fig. 5 Tumours in patients with Ma2 encephalitis. (**A**) Inflammatory infiltrates (right lower corner) and intratubular large, atypical cells in a patient with carcinoma *in situ* of the testis or intratubular germ-cell neoplasm. (**B**) This demonstrates that the atypical cells at the base of the seminiferous tubules are periodic acid–Schiff (PAS) positive, characteristic of intratubular germ-cell neoplasms; the same cells were placental alkaline phosphatase positive (not shown) a specific marker of neoplastic germ cells. (**C** and **D**) A seminoma and (**E** and **F**) an adenocarcinoma of the lung reacted with normal human IgG (**C** and **E**) and anti-Ma2 antibodies (**D** and **F**). Both tumours showed reactivity with anti-Ma2 IgG, indicating expression of Ma2 protein. **A**, haematoxylin and eosin, ×200; **B**, PAS counterstained with haematoxylin, ×100; **C–F**, counterstained with haematoxylin, ×200.

men younger than 45 years, with testicular tumours that had complete response to oncological treatment, limited involvement of the nervous system and absence of anti-Ma1 antibodies (Table 5). Although immunotherapy did not statistically associate with improvement, three of the patients that improved (patients 1, 8 and 9 in Table 4) developed neurological symptoms while the tumour was in remission for 6, 8 and 20 months, respectively. Patient 1 received immunotherapy and several symptomatic treatments for parkinsonism that could account for part of the improvement, but patient

8 received only IVIg and steroids resulting in dramatic clinical response. In this patient, discontinuation of immunotherapy resulted in neurological relapse that resolved after resuming treatment. At last follow-up, 22 months after neurological symptom development, the examination was normal and the patient was planning to return to work. Patient 9 received IVIg, steroids and anti-epileptic medication for treatment of isolated temporal lobe seizures with MRI evidence of limbic abnormalities and anti-Ma2 antibodies and oligoclonal bands in the CSF. This patient has been followed for 9 months; he is back at

Table 4 *Clinical features of patients who improved*

Patient no.	Age (years)/sex	Antibody	Tumour, stage	PND prior/after tumour diagnosis	Predominant syndrome	Additional features	Tumour treatment	Immunotherapy	Ranking prior/after treatment
1	28/M	Ma2	Testis limited	6 months after	Limbic-brainstem	Parkinsonism	Orchiectomy	Plasma exchange, steroids	4/2
2	26/M	Ma2	Testis, extensive	9 months prior	Limbic	None	Orchiectomy, chemotherapy	None	4/1
3	45/M	Ma2	Testis[b], limited	6 months prior	Brainstem	R > L truncal ataxia	Orchiectomy, radiation	None	4/3
4	30/M	Ma2	Testis[b], limited	26 months prior	Brainstem	R ataxia	Orchiectomy	None	4/2
5	26/M	Ma2	Testis, limited	7 months prior	Limbic	None	Orchiectomy, chemotherapy	None	4/3
6	67/M	Ma2	Lung, extensive	2 months prior	Limbic, diencephalic, brainstem	Gait ataxia	Surgery	Steroids, plasma exchange	3/2
7	26/M[a]	Ma2	Testis, limited	2 months prior	Brainstem	None	Orchiectomy, chemotherapy	IVIg, steroids	3/0
8	27/M[a]	Ma2	Testis, limited	8 months after	Limbic	None	Orchiectomy, chemotherapy	IVIg, steroids	3/0
9	33/M[a]	Ma2	Testis, limited	20 months after	Limbic	None	Orchiectomy, chemotherapy	IVIg, steroids	1/0
10	26/M	Ma2 and Ma1	Testis[c], limited	12 months prior	Limbic, diencephalic, brainstem	Opsoclonus narcolepsy	Orchiectomy	Steroids	4/3
11	35/M	Ma2	Testis[c], limited	16 months prior	Limbic, diencephalic, brainstem	Narcolepsy	Orchiectomy	Steroids, plasma exchange, cyclophosphamide	4/3

[a]Patients with complete neurological recovery. [b]Patients with pure seminoma; other patients with testicular tumours had non-seminomatous features. [c]Carcinoma *in situ* of the testis. M = male; F = female.

Table 5 *Neurological outcome and prognostic factors in 33 patients*

	Neurological improvement or stabilization (n = 18)	Neurological deterioration (n = 15)	P
Sex			
Male	15	8	0.07
Female	3	7	
Age			
≤45 years	14	6	0.03
>45 years	4	9	
Involvement of the CNS			
Unifocal*	10	1	0.003
Multifocal	8	14	
Anti-Ma reactivity			
Ma2	14	6	0.03
Ma2 and Ma1	4	9	
Tumour			
Testicular	14	4	0.004
Other	4	11	
Tumour response to treatment[†]			
Complete	16	5	0.001
Other[‡]	1	8	
Immunotherapy			
Yes	14	8	0.13
No	4	7	

*Of the 10 patients with unifocal involvement that improved or stabilized, six had limbic encephalitis, three brainstem encephalitis and one cerebellar degeneration. The patient with unifocal involvement who deteriorated had cerebellar degeneration. [†]Patients without identified tumour have been excluded from analysis. [‡]Partial response, no response or no treatment

work, free of seizures and the neurological examination is normal.

Among the other eight patients that improved, one had complete neurological recovery and seven partial but objective improvement (Table 4). One of these seven patients had almost complete recovery with normalization of memory, except for mild difficulty with abstraction (Burton *et al.*, 1988). One patient was able to return to driving, one to playing golf, one had a major reduction of dream-like episodes that used to last 60–90 min, one had partial improvement of brainstem encephalopathy and ataxia, and two had significant improvement of narcolepsy and confusion but both remained with stable memory deficits and one with seizures requiring multiple anti-epileptic treatment. Two of the patients that improved are now dead; one as a result of pulmonary embolism post-appendectomy 22 months after developing the PND. The other patient was able to return to most activities of daily living for 18 months, but eventually his symptoms recurred with an 11-month progression of brainstem deficits, memory loss and cognitive decline; tumour recurrence was identified shortly before his death.

Fifteen patients had steady deterioration; 12 are dead and three had neurological worsening at last follow-up. The median

follow-up of all these patients was 9 months (range 4 months to 4 years). Nine of the deceased patients died within the first year of the neurological disorder (median 6 months). The cause of death was neurological (eight), mixed progression of tumour and neurological deficits (two), complication of chemotherapy (one), and undetermined (one). Eight of the deceased patients (67%) had anti-Ma1 antibodies.

Clinical features in patients with anti-Ma1 and anti-Ma2 antibodies

Among the 15 patients with anti-Ma1 and anti-Ma2 antibodies (anti-Ma1 patients), 10 were women (age 53–82 years, median 63 years) and five men (age 23–58 years, median 39 years). In contrast, 21 of the 23 patients with only anti-Ma2 antibodies were men (age 22–70 years, median 23 years) and two women (age 65 and 72 years) ($P < 0.003$). This significant association between antibody group and gender, as well as the younger age of male patients, results from the robust association between the detection of anti-Ma2 antibodies in isolation and germ-cell tumours: 16 out of 23 patients with only anti-Ma2 antibodies had testicular germ-cell tumours (15 in testis), whereas only two of the 15 anti-Ma1 patients had testicular germ-cell tumours ($P = 0.0002$).

The main neurological findings in anti-Ma1 patients were brainstem dysfunction (11 patients), ataxia (nine), limbic encephalopathy (nine) and diencephalic dysfunction (three). Pure limbic or hypothalamic deficits were identified in two patients. When compared with patients with only anti-Ma2 antibodies, the anti-Ma1 patients were more likely to develop cerebellar dysfunction ($P = 0.009$); no significant differences were noted with respect to symptom presentation and other neurological features. Of 13 anti-Ma1 patients whose outcome is known, nine deteriorated, three stabilized and one improved. As indicated, anti-Ma1 patients had worse outcome than patients with only anti-Ma2 antibodies (Table 5; $P = 0.03$).

Discussion

We report the clinical features of 38 patients with encephalitis associated with anti-Ma2 antibodies. The majority of the patients developed symptoms of limbic, diencephalic or brainstem dysfunction with abnormal MRI findings. The importance of describing this disorder is two-fold. The combination of symptoms and predominant upper brainstem dysfunction resulted in a disorder that differs from classical paraneoplastic limbic or brainstem encephalitis, and is often unrecognized. More important, >50% of the patients, usually those with testicular germ-cell tumours, showed improvement or stabilization of neurological deficits after treating the cancer and immunosuppression.

We classified the patients into two clinical groups, one including 34 (89%) patients with isolated or combined symptoms of limbic, diencephalic or brainstem dysfunction, and a second group of four (11%) patients with other syndromes. Ataxia from involvement of the cerebellum, cerebellar

peduncles or brainstem occurred in 13 patients; it affected patients from both groups and in only five cases the deficits became as severe as in other types of paraneoplastic cerebellar degeneration. Overall, when considering the long-term follow-up or MRI findings in both clinical groups, 36 (95%) patients eventually developed involvement of the limbic system, diencephalon or brainstem.

Only 26% of patients with limbic encephalitis had a syndrome resembling the classical description of this disorder (Hierons *et al.*, 1978). Many of the other patients had associated EDS, eye movement abnormalities, or both. Early and prominent EDS occurred in 32% of patients of the entire series and 41% of those with limbic or diencephalic dysfunction. These patients differed from idiopathic EDS or narcolepsy in that all had accompanying limbic, brainstem or endocrine deficits. The EDS was likely due to hypothalamic dysfunction because the hypocretin levels were very low or undetectable in the CSF of patients with EDS but were normal in patients without EDS (Mignot *et al.*, 2002). Although follow-up of CSF hypocretin levels was not obtained, one of the patients with low levels at symptom presentation had important neurological improvement after tumour resection and steroids that lasted for 18 months, suggesting that low hypocretin levels do not imply irreversible hypothalamic dysfunction.

All 20 patients with brainstem symptoms associated with limbic or diencephalic deficits had prominent eye movement abnormalities, which in 60% resulted in VGP. While at the early stages vertical gaze deficits were usually overcome by oculocephalic and Bell's manoeuvres, at later stages the deficits became fixed, with frequent ptosis, skew deviation and horizontal gaze palsy. Furthermore, two of the five patients with a pure brainstem syndrome eventually developed VGP or MRI abnormalities in the superior colliculi and periaqueductal region. Long-term follow-up of the pupillary responses was not obtained, but most patients had normal pupillary responses to light by the time that the VGP and other ophthalmopareses were clearly established (data not shown). Overall, these findings suggest that in most patients with anti-Ma2 brainstem encephalitis, the disorder predominantly targets the upper brainstem structures involved in the supranuclear control of vertical gaze, followed during the course of the disease by involvement of the oculomotor nuclei, and less frequently horizontal gaze and abducens nuclei.

Three patients developed parkinsonism and two a syndrome that combined severe hypokinesia, decreased verbal output (one with mutism) and the appearance of extreme drowsiness. Although no autopsy was obtained from these patients, accompanying clinical features (four patients had VGP) or MRI findings suggested that the syndromes resulted from combined involvement of upper brainstem (all five patients), hypothalamus (two), medial thalami (two) and globus pallidus (one). Furthermore, the autopsy of three other patients who did not develop parkinsonism or hypokinesia showed inflammatory infiltrates or neuronophagia in the susbtantia nigra among other regions of the CNS. Overall, these findings suggest that patients with anti-Ma2 encephalitis are more likely to develop parkinsonian-hypokinetic features than patients with other PND.

Seventy-four percent of the patients had abnormal brain MRI; patients with limbic or diencephalic symptoms had more frequent abnormalities (89%). Despite these numbers, we believe that the frequency of MRI findings is underestimated, because follow-up MRIs were not systematically obtained. All eight patients (three with normal initial MRI) who had follow-up studies developed new abnormalities on the second MRI. Furthermore, new MRI technology has likely improved the diagnosis; 19 out of 20 patients diagnosed since 1999 had abnormal findings on the initial MRI. Thirty-six percent of patients with abnormal MRI had contrast enhancement; some of the abnormalities resembling a tumour mass, as reported previously (Rosenfeld *et al.*, 2001).

Even though the clinical and radiological findings of anti-Ma2 encephalitis suggest a disorder that predominantly targets the limbic system-diencephalon-upper brainstem, and sometimes the cerebellum, the distribution of abnormalities in the autopsy of four patients was more widespread. It is important to note that all patients who underwent autopsy studies had anti-Ma1 and anti-Ma2 antibodies, the former associated with more extensive brainstem or cerebellar involvement and carrying a poorer prognosis, as demonstrated in this study. Notwithstanding, none of the 38 patients developed a syndrome similar to the paraneoplastic sensory neuronopathy and encephalomyelitis or autonomic dysfunction associated with anti-Hu or CV2/CRMP5 (collapsin response mediator protein) antibodies (Honnorat *et al.*, 1996; Antoine *et al.*, 2001; Graus *et al.*, 2001).

Among the 34 patients with cancer, 53% had testicular germ-cell tumours and 47% other cancers, with non-small cell lung cancer (non-SCLC) the most prevalent. The fact that two patients had carcinoma *in situ* of the testis that was revealed at the pre-invasive stage by the detection of anti-Ma2 antibodies and new abnormalities appearing in serial ultrasound studies, suggests that the immune response is triggered in the testis at the early stages of cancer. In support of this hypothesis, both patients had inflammatory infiltrates in the vicinity of the neoplastic cells, and a third patient developed a transient episode of 'epididymo-orchitis' that preceded by several weeks the development of PND and by 5 months the detection of a seminoma, likely representing the early immunological response at the tumour site. These two patients, along with 13 additional patients with testicular neoplasms, had the tumour limited to the testis at the time of diagnosis, allowing complete surgical resection of the neoplasm. This is in contrast to the paraneoplastic syndromes associated with anti-Hu or anti-Yo antibodies, in which most patients have metastatic invasion of the regional lymph nodes by the time of tumour diagnosis. For example, in a series of 200 patients with anti-Hu-associated encephalomyelitis or sensory neuronopathy, the usual tumour location was in the mediastinal lymph nodes and 25% had systemic metastases (Graus *et al.*, 2001). In a series of 30 patients with anti-Yo-associated cerebellar degeneration, all 12 patients with breast cancer had metastatic lymph nodes and 15 out of 18 patients with

ovarian cancer had metastases at the time of tumour diagnosis (Rojas *et al.*, 2000).

Of the four patients without a tumour diagnosis, two might have had an occult cancer or experienced tumour regression. Both had testicular microcalcification demonstrated in pathological studies (one orchiectomy, one autopsy) and one had a history of cryptorchidism until he was 10 years old. Cryptorchidism and microcalcification are independent risk factors for germ-cell tumours; orchiectomy is recommended for undescended testis after 2 years of age (Berkmen and Alagol, 1998), and tubular microcalcification coexists with tumour in 30–40% of the patients (Derogee *et al.*, 2001). We searched for cells expressing Ma proteins in the orchiectomy of one of the patients, but the testis had been replaced by fibrous tissue and no reactive cells were found (data not shown).

As far as the clinical outcome is concerned, 33% of the patients had neurological improvement and 21% long-term symptom stabilization. Patients that improved or stabilized often received immunotherapy, but in most instances the clinical course did not change until the tumour was treated. In two patients, neurological relapse heralded tumour recurrence; in one the tumour was re-treated and resulted in neurological improvement, supporting the importance of treating the underlying cancer. Patients with testicular tumours that had complete response to therapy were more likely to improve neurologically than the other patients. Neurological improvement is rarely seen in other paraneoplastic syndromes associated with tumours that are more difficult to treat, such as SCLC, breast or gynaecological cancers. Only four out of 200 (2%) patients with anti-Hu-associated encephalomyelitis had improvement of CNS symptoms, and none of 34 patients with anti-Yo-associated cerebellar degeneration had neurological improvement after oncological treatment, immunotherapy or both (Rojas *et al.*, 2000; Graus *et al.*, 2001).

Despite the lack of a statistical association between neurological improvement and immunotherapy, there are two findings that suggest that immunotherapy contributes to improvement. First, patients who only received immunosuppression were more likely to improve that those who did not receive any treatment. Secondly, in one patient neurological relapse resolved after resuming immunotherapy.

The presence of anti-Ma1 antibodies carried a worse prognosis. In these patients the more extensive involvement of the brainstem and cerebellum could account for the poor outcome. However, a more important factor could be that 80% of the patients had non-testicular cancers which, as occurs with the tumours associated with other paraneoplastic immunities, are more difficult to treat than testicular germ-cell tumours.

Most recent studies on PND focus on the search and testing of onconeural antibodies but overlook the degree of syndrome specificity or the clinical details of the associated syndromes. The current study demonstrates that anti-Ma2 antibodies (with or without anti-Ma1 antibodies) define a disorder with remarkable involvement of the limbic system, diencephalon and brainstem. The disorder should be suspected in patients with symptoms of involvement of one or more of these three brain regions, and clinical progression for weeks or months (usually <6 months), MRI findings in medial temporal lobes, hypothalamus, basal ganglia, thalami or upper brainstem-collicular region, and CSF abnormalities suggesting an inflammatory process. Because there is frequent involvement of the diencephalon and upper brainstem, EDS and vertical ophthalmoparesis are common. In young male patients (<45 years) the primary tumour is usually in the testis; in other patients the repertoire of tumours is varied, but the leading neoplasm is non-SCLC. The importance of recognizing the syndrome is that patients may improve with prompt identification and treatment of the tumour and immunosuppression.

References

Antoine JC, Honnorat J, Camdessanche JP, Magistris M, Absi L, Mosnier JF, et al. Paraneoplastic anti-CV2 antibodies react with peripheral nerve and are associated with a mixed axonal and demyelinating peripheral neuropathy. Ann Neurol 2001; 49: 214–21.

Barnett M, Prosser J, Sutton I, Halmagyi GM, Davies L, Harper C, et al. Paraneoplastic brain stem encephalitis in a woman with anti-Ma2 antibody. J Neurol Neurosurg Psychiatry 2001; 70: 222–5.

Bennett JL, Galetta SL, Frohman LP, Mourelatos Z, Gultekin SH, Dalmau JO, et al. Neuro-ophthalmologic manifestations of a paraneoplastic syndrome and testicular carcinoma. Neurology 1999; 52: 864–7.

Berkmen F, Alagol H. Germinal cell tumors of the testis in cryptorchids. J Exp Clin Cancer Res 1998; 17: 409–12.

Burton GV, Bullard DE, Walther PJ, Burger PC. Paraneoplastic limbic encephalopathy with testicular carcinoma. A reversible neurologic syndrome. Cancer 1988; 62: 2248–51.

Dalmau J, Gultekin SH, Voltz R, Hoard R, DesChamps T, Balmaceda C, et al. Ma1, a novel neuron- and testis-specific protein, is recognized by the serum of patients with paraneoplastic neurological disorders. Brain 1999; 122: 27–39.

Derogee M, Bevers RF, Prins HJ, Jonges TG, Elbers FH, Boon TA. Testicular microlithiasis, a premalignant condition: prevalence, histopathologic findings, and relation to testicular tumor. Urology 2001; 57: 1133–7.

Dropcho EJ. Neurologic paraneoplastic syndromes. Curr Oncol Rep 2004; 6: 26–31.

Graus F, Keime-Guibert F, Reñé R, Benyahia B, Ribalta T, Ascaso C, et al. Anti-Hu-associated paraneoplastic encephalomyelitis: analysis of 200 patients. Brain 2001; 124: 1138–48.

Gultekin SH, Rosenfeld MR, Voltz R, Eichen J, Posner JB, Dalmau J. Paraneoplastic limbic encephalitis: neurological symptoms, immunological findings and tumour association in 50 patients. Brain 2000; 123: 1481–94.

Henson RA, Urich HE, editors. Cancer and the nervous system. Oxford: Blackwell Scientific; 1982. p. 314–45.

Hierons R, Janota I, Corsellis JA. The late effects of necrotizing encephalitis of the temporal lobes and limbic areas: a clinico-pathological study of 10 cases. Psychol Med 1978; 8: 21–42.

Honnorat J, Antoine JC, Derrington E, Aguera M, Belin MF. Antibodies to a subpopulation of glial cells and a 66 kDa developmental protein in patients with paraneoplastic neurological syndromes. J Neurol Neurosurg Psychiatry 1996; 61: 270–8.

Landolfi JC, Nadkarni M. Paraneoplastic limbic encephalitis and possible narcolepsy in a patient with testicular cancer: case study. Neuro-oncology 2003; 5: 214–6.

Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, et al. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002; 59: 1553–62.

Overeem S, Dalmau J, Bataller L, Nishino S, Mignot E, Verschuuren J, et al. Anti-Ma2 antibodies in idiopathic and paraneoplastic hypocretin-deficient narcolepsy. Neurology 2004; 62: 138–40.

Posner JB, editor. Neurologic complications of cancer. Philadelphia: F. A. Davis; 1995. p. 353–85.

Rojas I, Graus F, Keime-Guibert F, Reñé R, Delattre JY, Ramón JM, et al. Long-term clinical outcome of paraneoplastic cerebellar degeneration and anti-Yo antibodies. Neurology 2000; 55: 713–5.

Rosenfeld MR, Eichen JG, Wade DF, Posner JB, Dalmau J. Molecular and clinical diversity in paraneoplastic immunity to Ma proteins. Ann Neurol 2001; 50: 339–48.

Sutton I, Winer J, Rowlands D, Dalmau J. Limbic encephalitis and antibodies to Ma2: a paraneoplastic presentation of breast cancer. J Neurol Neurosurg Psychiatry 2000; 69: 266–8.

Voltz R, Gultekin SH, Rosenfeld MR, Gerstner E, Eichen J, Posner JB, et al. A serologic marker of paraneoplastic limbic and brain-stem encephalitis in patients with testicular cancer. N Engl J Med 1999; 340: 1788–95.

Wong AM, Musallam S, Tomlinson RD, Shannon P, Sharpe JA. Opsoclonus in three dimensions: oculographic, neuropathologic and modelling correlates. J Neurol Sci 2001; 189: 71–81.