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Encephalitis lethargica syndrome: 20 new cases and evidence of basal ganglia autoimmunity

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Summary

In 1916, von Economo first described encephalitis lethargica (EL), a CNS disorder presenting with pharyngitis followed by sleep disorder, basal ganglia signs (particularly parkinsonism) and neuropsychiatric sequelae. Since the 1916–1927 epidemic, only sporadic cases have been described. Pathological studies revealed an encephalitis of the midbrain and basal ganglia, with lymphocyte (predominantly plasma cell) infiltration. The EL epidemic occurred during the same time period as the 1918 influenza pandemic, and the two outbreaks have been linked in the medical literature. However, von Economo and other contemporary scientists thought that the 1918 influenza virus was not the cause of EL. Recent examination of archived EL brain material has failed to demonstrate influenza RNA, adding to the evidence that EL was not an invasive influenza encephalitis. By contrast, the findings of intrathecal oligoclonal bands (OCB) and beneficial effects of steroid treatments have provoked the hypothesis that EL may be immune-mediated. We have recently seen 20 patients with a similar EL phenotype, 55% of whom had a preceding pharyngitis. The patients had remarkable similarity to the historical descriptions of EL: sleep disorder (somnolence, sleep inversion or insomnia), lethargy, parkinsonism, dyskinesias and neuropsychiatric symptoms. CSF examination commonly showed elevated protein and OCB (75 and 69% respectively). Investigation found no evidence of viral encephalitis or other recognized causes of rapid-onset parkinsonism. MRI of the brain was normal in 60% but showed inflammatory changes localized to the deep grey matter in 40% of patients. We investigated the possibility that this phenotype could be a postinfectious autoimmune CNS disorder, and therefore similar to Sydenham's chorea. Anti-streptolysin-O titres were elevated in 65% of patients. Furthermore, western immunoblotting showed that 95% of EL patients had autoantibodies reactive against human basal ganglia antigens. These antibodies were also present in the CSF in four patients tested. By contrast, antibodies reactive against the basal ganglia were found in only 2–4% of child and adult controls (n = 173, P < 0.0001). Rather than showing polyspecific binding, these antibodies bound to common neural autoantigens of molecular weight 40, 45, 60 and 98 kDa. Regional tissue comparisons showed that the majority of these autoantigens were specific to or enriched in CNS tissue. Immunohistochemistry with secondary staining localized antibody binding to neurons rather than glial populations. Further investigation is required to determine whether these antibodies affect neuronal function (i.e. whether they are pathogenic anti-neuronal antibodies). Histopathology in one case demonstrated striatal encephalitis with perivenous B- and T-lymphocytic infiltration. We believe an EL-like syndrome is still prevalent, and propose that this syndrome may be secondary to autoimmunity against deep grey matter neurons.

Keywords: autoimmune encephalitis; Sydenham's chorea; parkinsonism

Abbreviations: ASOT = anti-streptolysin-O titre; EL = encephalitis lethargica; GFAP = glial fibrillary acidic protein; OCB = oligoclonal band(s); PANDAS = paediatric autoimmune neuropsychiatric disorders associated with streptococcal infections

Introduction

Lethargic encephalitis has been known for many centuries, having been described by physicians such as Hippocrates and Sydenham. The most recent epidemic ravaged the world between 1916 and 1927, and was named encephalitis lethargica (EL) by the clinician most associated with this disorder, von Economo (von Economo, 1931). Other than sleep disturbance, lethargy and extrapyramidal movements, von Economo noted that neuropsychiatric disorders were common in the survivors (e.g. catatonia, obsessive–compulsive disorder and mutism). Additional common features included oculogyric crises, ocular features (ophthalmoplegia and ptosis) and central cardiorespiratory features (particularly hiccough). Pathological findings, like the clinical characteristics, localized the encephalitis to basal ganglia and midbrain structures.

Because EL was epidemic during the same time period as the 1918 influenza pandemic, an association between the two diseases has been proposed (Ravenholt and Foege, 1982). However, von Economo and other workers during the EL epidemic thought that influenza was not the cause. The recent discovery and identification of the 1918 influenza virus by Taubenberger and colleagues (Taubenberger *et al*., 1997) allowed this group the opportunity to examine archived EL brain specimens for 1918 influenza virus RNA. They failed to find influenza RNA in EL brains, and furthermore determined that the 1918 influenza virus was genetically incapable of neurotropic disease, and only capable of reproduction in the respiratory tree. The authors concluded that EL was unlikely to have been directly due to the 1918 influenza virus (McCall *et al*., 2001).

There have been no further epidemics of EL since the 1920s, although sporadic cases have continued to be reported (Rail *et al*., 1981; Howard and Lees, 1987; Blunt *et al*., 1997; Kiley and Esiri, 2001). Recent reports have consistently failed to demonstrate evidence of neurotropic viral particles, and the cause of EL remains unknown. However, the finding of CSF oligoclonal bands (OCB) (Williams *et al*., 1979; Howard and Lees, 1987; Kiley and Esiri, 2001) and the successful treatment of some recent cases with steroids (Blunt *et al*., 1997) have led investigators to propose that this phenotype may be immune-mediated. Over the last 3 years, we have observed a number of children and adults with an EL-like syndrome, often following pharyngeal infections. Group A *Streptococcus* is the commonest cause of pharyngitis, and is a recognized cause of immune-mediated (autoantibody) basal ganglia dysfunction, the classical phenotype being Sydenham's chorea (Husby *et al*., 1976; Church *et al*., 2002). We therefore examined these EL-like patients for evidence of autoreactive antibodies against the basal ganglia.

Material and methods

Patients

All patients were referred to tertiary neurology centres between April 1999 and May 2002 with a new-onset CNS dysfunction resulting in an EL-like syndrome (sleep disorder and associated lethargy, parkinsonism and neuropsychiatric disorders). Other than 'high-functioning' autism (n = 1) and Down's syndrome (n = 1), there was no past medical history of note. Movement disorders were video-recorded and reviewed by experienced child neurologists (R.A.H.S., B.G.N.). Psychiatric disturbance was often acute, with Diagnostic and Statistical Manual of Mental Disorders—Fourth Edition (DSM-IV) criteria only possible after the acute phase (DSM-IV diagnoses are specified in the text).

Controls

To determine the specificity of anti-basal ganglia antibodies and streptococcal serology, the following controls were used for comparison (demographics are shown in Table 1): children with dystonia of alternative aetiologies (n = 33), children with recent (within 4 weeks) uncomplicated group A streptococcal infection (n = 40), adults with mixed neurological disease (n = 50), and healthy adults (n = 50). Controls were recruited during the same time period as the patients.

Investigations

Patients were investigated by the responsible clinician according to the patient's mode of presentation (Table 2). Evidence of recent streptococcal infection was assessed using anti-streptolysin–O titre (ASOT; Dade Behring II nephelometry). The World Health Organization guideline for normal ASOT is <200 IU/ml. All patients with elevated streptococcal titres had convalescent serology 6 months later, which showed a reduction in titres.

Table 1 *Demographics of patient and control groups*

Group	EL	Children with uncomplicated GAS infection	Children with dystonia	Adults with mixed neurological disease	Healthy adults
Number	20	40	33	50	50
Sex: M, F	11, 9	25, 15	17, 16	30, 20	25, 25
Mean (range) age (years)	Child: 10.4 (2–16) Adult: 47.5 (17–69)	9.8 (2–15)	9.1 (1–16)	41.1 (19–70)	35.6 (19–57)

Children with dystonia control group (n = 33) included the following aetiologies: encephalitis (n = 13); basal ganglia stroke (n = 7); neurometabolic disease (n = 6); miscellaneous (n = 7). GAS = group A streptococcus; M = male; F = female.

Anti-neuronal antibodies

Human basal ganglia western immunoblotting

The anti-basal ganglia antibody method has been shown to be a sensitive and specific marker in Sydenham's chorea and is described in detail elsewhere (Church *et al*., 2002). All anti-basal ganglia antibody tests were performed by A.J.C., who was blinded to the clinical details. The caudate, putamen and globus pallidus from a human donor with no evidence of neurological disease were kindly provided by the Queen Square Brain Bank for neurological disorders. Brains from different donors have been compared previously and show little inter-assay variation. The block of tissue was homogenized with a small volume of saline containing protease inhibitors (Sigma, Poole, UK) and centrifuged for 30 min at 12 000 rev/min. Equal volumes of supernatant and di-isopropyl ether were mixed and centrifuged at 3000 rev/min for 10 min to remove lipid from the supernatant. The protein fraction was then collected and stored at –80°C until required. It is acknowledged that samples prepared by this method will contain mainly soluble proteins, and lipophilic proteins are less likely to be present in this supernatant fraction.

The homogenate was mixed with lithium dodecyl sulphate sample buffer (Invitrogen, USA) containing 0.05 M dithiothreitol, and heated at 65°C for 15 min. A total of 30 μg of protein was loaded onto a 4–12% Bis-Tris gel (Invitrogen) and electrophoresed. The proteins were blotted onto nitrocellulose (Sartorius, Epsom, UK) and blocked with 2% milk proteins for 2 h. Total serum immunoglobulin (Ig) G was measured in five random EL patients and was within the normal range in all. Samples were diluted 1/300, applied to the blot and incubated overnight at 4°C. The nitrocellulose was washed with 10 changes of 0.9% saline containing 0.2% milk proteins and 0.025% Tween. The blot was incubated for 2 h with rabbit anti-human IgG conjugated with horseradish peroxide diluted 1/1000 (Dako, Cambridge, UK). After washing, the substrate 4-chloro-1-naphthol (Sigma) was added and the blot was allowed to develop for 15 min.

Rat brain western immunoblotting

Brain homogenate from adult male Wistar rats was prepared in the same way as the human basal ganglia method. Unlike the human basal ganglia method, 90 μg of protein was loaded onto each 2-D gel for transfer to nitrocellulose. Serum dilutions, secondary antibody and antibody detection were the same as in the human basal ganglia method. When comparing CSF with serum for anti-neuronal antibodies, serum was diluted 400-fold compared with CSF, in accordance with the recognized differences in CSF/serum IgG concentrations. In the CSF/serum comparisons, the secondary antibody conjugated with horseradish peroxide (Dako) was diluted 1 : 5000 and developed using enhanced chemiluminescence (Pierce, USA).

Regional localization of autoantigens in rat tissue

Likewise, rat brain (cerebellum removed), cerebellum, liver, kidney and heart were homogenized and prepared as in the human basal ganglia method. Total proteins were measured using the biuret method (Bio-Rad, UK). For the regional comparisons, the total proteins from each tissue were normalized and loaded onto 10-lane gels. Serum dilutions, secondary antibody and antibody detections were the same as in the human basal ganglia method.

Human basal ganglia indirect immunofluorescence

Sections (10 μm) of normal human basal ganglia (caudate, putamen and midbrain) were cut from snap-frozen tissue. Control and patient serum was diluted 1 : 25 in phosphate-buffered saline and overlaid onto prepared slides and incubated for 30 min (1 : 50 for midbrain due to higher background fluorescence). A control slide was also prepared and incubated with phosphate-buffered saline for 30 min to assess background fluorescence. All slides were washed in phosphate-buffered saline and incubated with anti-human IgG conjugated with fluorescein isothiocyanate (Dako) and analysed using fluorescence microscopy. To identify cell populations involved in human antibody binding, we used commercially available antibodies. Glial cell populations were labelled with a monoclonal antibody against human glial fibrillary acidic protein (GFAP; mouse anti-human; Sternberg Monoclonals). Neuronal cells were labelled using a monoclonal antibody against human neuron-specific enolase (mouse anti-human; Abcam, UK). Anti-mouse IgG conjugated with fluorescein isothiocyanate was used for detection (Dako). To assess the identity of cell population involved in anti-basal ganglia antibody binding, we double-stained human caudate/putamen sections with patient IgG and commercial GFAP antibody.

Table 2 *Negative or normal investigations in EL patients*

Aetiology	Diagnostic investigation
Infectious	CSF herpes simplex virus PCR (n = 12), CSF enterovirus PCR (n = 5), CSF mycoplasma PCR (n = 9), CSF Epstein–Barr virus PCR (n = 8), CSF measles antibody (n = 4), CSF bacterial culture (n = 16), mycoplasma serology (n = 9), Lyme serology (n = 6), Whipple's disease, varicella zoster, influenza, arbovirus, picorna, HHV6, HHV7, adenovirus, parvovirus, bartonella, HIV (all n = 2)
Systemic autoimmune disease	Anti-nuclear antibody* (n = 16), complement (n = 5), angiotensin-converting enzyme (n = 6), anti-cardiolipin antibodies (n = 3)
Metabolic	CSF lactate (n = 14), creatine kinase (n = 4), ammonia (n = 4), plasma amino acids (n = 7), urinary organic acids (n = 8), very long chain fatty acids (n = 4), white cell enzymes (n = 6), liver function tests (n = 10), acanthocytes (n = 6)
Biochemical	Copper (n = 12), caeruloplasmin (n = 9), thyroid function tests (n = 10), porphyrin screen (n = 3), calcium metabolism (n = 16), urine toxicology (n = 7)
Genetic	Huntington's disease (n = 3), DRPLA (n = 3), SCA-3 (n = 2), DYT-1 (n = 4)

Investigations were tailored to the patient's age, presentation and course (number in parentheses). *Anti-nuclear antibody was <1 : 80 in all patients. DRPLA = dentatorubral pallidoluysian atrophy; SCA-3 = spinocerebellar ataxia type 3; DYT-1 = DYT-1 dystonia.

Patient IgG was detected with anti-human IgG conjugated with tetramethyl rhodamine isothiocyanate (Dako). GFAP was detected using anti-mouse IgG conjugated with fluorescein isothiocyanate, and assessed using a fluorescence microscope with dual filters.

Statistics

The ASOT data were compared using the Wilcoxon two-sample test. The anti-basal ganglia antibody western immunoblotting data were compared using the χ^2 test.

Results

Clinical features

Patient demographics

The demographics and clinical characteristics of the patients with the EL phenotype are presented in Table 3.

Preceding infection and tempo of presentation

Eleven patients (55%) had an infection shortly before disease onset. The infections were described as upper respiratory tract infection (n = 6) and tonsillitis (n = 5). Presentation was described as acute, subacute and insidious in 35, 50 and 15% of patients respectively.

Sleep disturbance and lethargy

Nineteen of 20 (95%) patients had sleep disturbance, hypersomnolence (n = 12), insomnia (n = 2) or sleep inversion (n = 5). It was usually possible to rouse the somnolent patients without difficulty, but they would fall asleep if not stimulated. Other sleep abnormalities included vivid nightmares (n = 2) and sleep-walking (n = 1). Ten patients had lethargy, which was in excess of what would be expected for the degree of motor weakness.

Parkinsonism

All patients had signs consistent with parkinsonism. Twelve of 20 patients had a Parkinson's syndrome according to United Kingdom Parkinson's Disease Society Brain Bank criteria: six had all three cardinal features (bradykinesia, rigidity and rest tremor), two had bradykinesia with postural instability, two had bradykinesia with rigidity, and two had bradykinesia with rest tremor. The other eight patients had isolated features of the parkinsonian phenotype: bradykinesia/akinesia (n = 6), rest tremor (n = 1) or rigidity with rest tremor (n = 1).

Dyskinesias

In addition to parkinsonism, 11 patients had evidence of dyskinetic movement disorders: dystonia (n = 6, of whom five had generalized dystonia); chorea/hemiballismus (n = 2); motor tics (n = 2); stereotypies (n = 2); facial grimacing and blepharospasm (n = 1 each). Three patients had oculogyric crises.

Psychiatric disturbance

The psychiatric manifestations are described in Table 3. Seventeen of 20 patients had psychiatric disturbance. Mutism occurred in ten patients. Emotional disorders were also common and included depression DSM-IV (n = 6), obsessive–compulsive disorder DSM-IV (n = 3) and anxiety (n = 2). Apathy and catatonia occurred in four and three patients respectively.

Other features

Five patients had profound reduction in consciousness and required ventilation. Eight patients had ocular abnormalities [four ophthalmoplegia, three pupillary disturbance (one with ophthalmoplegia), one ptosis, one optic neuritis]. Hyperventilation and nocturnal bradycardia were also observed (Table 3). Seizures and memory loss occurred in three patients each. Symptoms of intracranial pathology were common, including headache (n = 6), photophobia (n = 3) and meningism (n = 2). Other features included incontinence (n = 3) and limb pains (n = 2).

Course and outcome

Ten patients had a monophasic illness, seven had a relapsing polyphasic course, two had static disease and one had progressive disease until death. After a mean follow-up of 5 months (range 2–14 months), only five patients have made a complete recovery to date. Of the 15 patients with continuing impairments, six have a persisting movement disorder and ten have disabling neuropsychiatric disturbance (one patient has both movement and psychiatric disorders).

Case example 1

A 15-year-old boy presented with acute paranoia. There was no preceding infection. After 3 days, he developed paroxysmal eye deviation, contortion of the tongue and abnormal dystonic limb posturing with retained awareness. These oculogyric and dystonic crises occurred up to 10 times per day. Over the following 2 weeks, he became increasingly agitated, paranoid and mute. He suffered extreme motor restlessness, dystonic posturing, intractable blinking, and compulsive touching of his body. He became progressively more bradykinetic with a stooped gait and developed rest tremor. There was no rigidity. His sleep pattern was inverted, with daytime somnolence and nocturnal insomnia. Positive investigations included intrathecal synthesis of OCB and elevated CSF protein. PCR of CSF for neurotropic viruses was negative. After a month of deterioration, his disease course has stabilized; 8 months into his illness he remains mute and agitated, and requires ongoing in-patient psychiatric care.

Table 3 *Patient demographics and clinical characteristics*

Age and sex	Sleep disorder	Lethargy	Parkinsonism	Dyskinesia	Psychiatric	Other features
2, F	Hypersomnolence	Absent	Bradykinesia, rigidity, rest tremor	–	–	–
4, M	Hypersomnolence	Present	Bradykinesia, rigidity, rest tremor	–	Emotional lability	Encephalopathy, meningism
5, M	Hypersomnolence	Present	Bradykinesia, rigidity	–	Mutism	Ophthalmoplegia, ptosis, nocturnal bradycardia
7, F	Sleep inversion	Present	Bradykinesia	Dystonic jaw	Mutism, anxiety, depression, apathy	Hyperventilation
8, M	Insomnia	Absent	Bradykinesia, postural instability	Generalized dystonia	Mutism, agitation, poor social interaction	Encephalopathy, seizures
9, F	Sleep inversion	Absent	Bradykinesia, rigidity	Oculogyric crises, hemiballismus	Mutism, disinhibition, compulsive touching	Ophthalmoplegia, hiccough
10, F	Hypersomnolence	Present	Bradykinesia	–	Aggression, depression, apathy	–
10, M	Hypersomnolence	Absent	Bradykinesia	Motor tics	Mutism, depression	–
11, F	Insomnia	Absent	Rest tremor	Facial grimacing	Catatonia, agitation, panic attacks	Seizures
13, F	Hypersomnolence	Present	Bradykinesia, postural instability	–	Apathy	Ophthalmoplegia, meningism, optic neuritis
13, M	Hypersomnolence	Absent	Bradykinesia	Generalized dystonia	Mutism, confusion	Encephalopathy, memory loss
14, F	Hypersomnolence	Present	Akinesia	Blepharospasm	Mutism, depression, auditory hallucinations	Pupillary light abnormality
15, F	Hypersomnolence	Present	Bradykinesia, rest tremor	Dystonic posturing	Depression	Memory loss
15, M	Sleep inversion	Absent	Bradykinesia, rest tremor	Oculogyric crises, dystonia	Mutism, catatonia, compulsions	–
15, M	Hypersomnolence	Present	Bradykinesia, rigidity, rest tremor	Oculogyric crises	Anxiety, paranoia	Pupillary light abnormality, hiccough
16, M	–	Absent	Bradykinesia, rigidity, rest tremor	–	–	Hyperventilation
17, M	Sleep inversion	Absent	Bradykinesia	Motor and vocal tics, stereotypies	Mutism, catatonia, obsessive–compulsive disorder, trichillomania	Hyperventilation
35, M	Hypersomnolence	Present	Bradykinesia, rigidity, rest tremor	–	Obsessive–compulsive disorder, depression	Ophthalmoplegia
69, M	Sleep inversion	Present	Bradykinesia, rigidity, rest tremor	Stereotypies	Mutism, apathy, confusion	Encephalopathy, pinpoint pupils
69, F	Hypersomnolence	Absent	Rigidity, rest tremor	Dystonia, chorea	–	Encephalopathy, memory loss, Seizures

M = male; F = female; OCD = obsessive–compulsive disorder.

Case example 2

A 15-year-old boy presented with an acute personality change 10 days after an upper respiratory tract infection. He became extremely anxious and worried about his safety. One week later he had an oculogyric crisis and developed upper-limb resting tremor and bradykinesia. This was followed by extreme daytime somnolence, lethargy and intractable hiccough. On examination, he would fall asleep if not stimulated and yawned continuously. Pupillary responses were poorly reactive to light and accommodation. There was tongue tremor, a positive glabellar tap and slow speech. Limb examination revealed rigidity with cogwheeling, bradykinesia and freezing. He had a stooped gait with poor arm-swing. Positive results included an elevated ASOT (350 IU/ml) and a mirrored pattern of OCB in both CSF and serum. PCR of CSF for neurotropic viruses was negative. MRI of the brain showed enhancement of the basal ganglia. He was treated with 50 mg of levodopa twice a day (with carbidopa), which improved his sleep disorder and parkinsonian signs, although he complained of insomnia. His abnormal clinical signs remained for 2 months, following which the levodopa was withdrawn. He has made a complete recovery with no neurological or psychiatric sequelae at 1 year of follow-up.

CSF and neuroimaging findings

CSF was examined in 16 patients and was abnormal in 13. There was a raised lymphocyte cell count in two patients. Twelve patients had an elevated protein concentration [>0.3 g/dl in children, >0.45 g/dl in adults (mean CSF protein of the 16 EL patients 0.55, range 0.13–1.2 g/dl)]. Paired isoelectric focusing in CSF and serum was performed in 13 patients; an intrathecal OCB pattern was present in 5/13, a mirrored pattern of OCB in both CSF and serum in 4/13, and no OCB in 4/13.

MRI was performed in all patients and was abnormal in eight (40%). All abnormal images had high signal lesions on T2 imaging. The abnormalities were present in the basal ganglia (n = 8), midbrain/tegmentum (n = 5), thalamus (n = 2), cerebral peduncle (n = 1) and temporal lobe (n = 1) (Fig. 1). Three patients had convalescent imaging during the recovery phase of the illness, which showed resolution of the abnormalities (Fig. 1d).

Aetiological investigations

Investigations were tailored to the age of the patient and presentation characteristics. Negative investigations are presented in Table 2. Two patients had concomitant tonsillitis; group A β-haemolytic *Streptococcus* was grown from the throat in both patients. ASOT was determined in all patients and controls. The data are presented in Table 4. The mean ASOT in the EL-like cohort was significantly elevated compared with the child dystonia ($P < 0.005$), adult neurology ($P < 0.0001$) and adult healthy controls ($P < 0.0001$). ASOT was elevated in 65% of EL patients but normal in 35%. There was no statistical difference between the EL group and the child streptococcal group ($P = 0.17$)

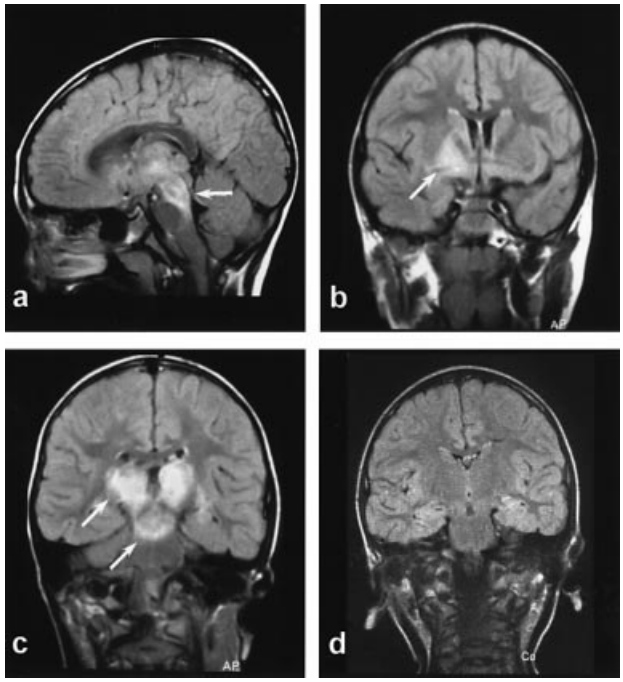

Fig. 1 MRI of the brain in a somnolent patient with bradykinesia and rigidity, showing inflammatory lesions (arrows) in the (**a**) midbrain and periaqueductal grey matter, (**b**) right putamen and (**c**) bilateral thalami and midbrain. (**d**) Convalescent imaging showing resolution of the inflammatory changes in the thalami and midbrain.

Anti-neuronal antibodies

Human basal ganglia western immunoblotting

Western immunoblotting allows discrimination of discrete antibody–antigen binding from polyspecific binding. The patient and control group findings are presented in Table 4. Positive binding using western immunoblotting was statistically more prevalent in the EL group compared with all controls ($P < 0.0001$ for each control group). The basal ganglia autoantigens are the same as those observed in Sydenham's chorea (40, 45 and 60 kDa) (Church *et al*., 2002), although the 98 kDa autoantigen appeared to be specific to this EL phenotype.

Rat brain western immunoblotting

To determine whether the basal ganglia autoantigens were also present in rat brain, we electroblotted both human basal ganglia and rat brain homogenates, and then incubated serum diluted 1 : 300. Patients with strong autoantibody reactivity against each of the autoantigens were selected. All of the autoantigens (40, 45, 60, 98 kDa) are common to both human basal ganglia and rat brain (example given in Fig. 2a).

Table 4 *Streptococcal serology and anti-basal ganglia antibodies*

Group	EL	Child streptococcus controls	Child dystonia controls	Adult neurology controls	Adult healthy controls
Mean ASOT (95% confidence interval) (IU/ml)	301 (198–404)	332 (269–395)	151 (99–203)	133 (98–168)	122 (101–143)
Positive for anti-basal ganglia antibodies on western immunoblotting	19/20 (95%)	1/40 (2.5%)	1/33 (3%)	1/50 (2%)	2/50 (4%)
Molecular weight of basal ganglia antigen (western immunoblotting) (kDa)	35 (n = 2) 40 (n = 11) 45 (n = 6) 60 (n = 11) 62 (n = 2) 80 (n = 2) 98 (n = 7)	60 (n = 1)	43 (n = 1)	40 (n = 1)	55 (n = 2)

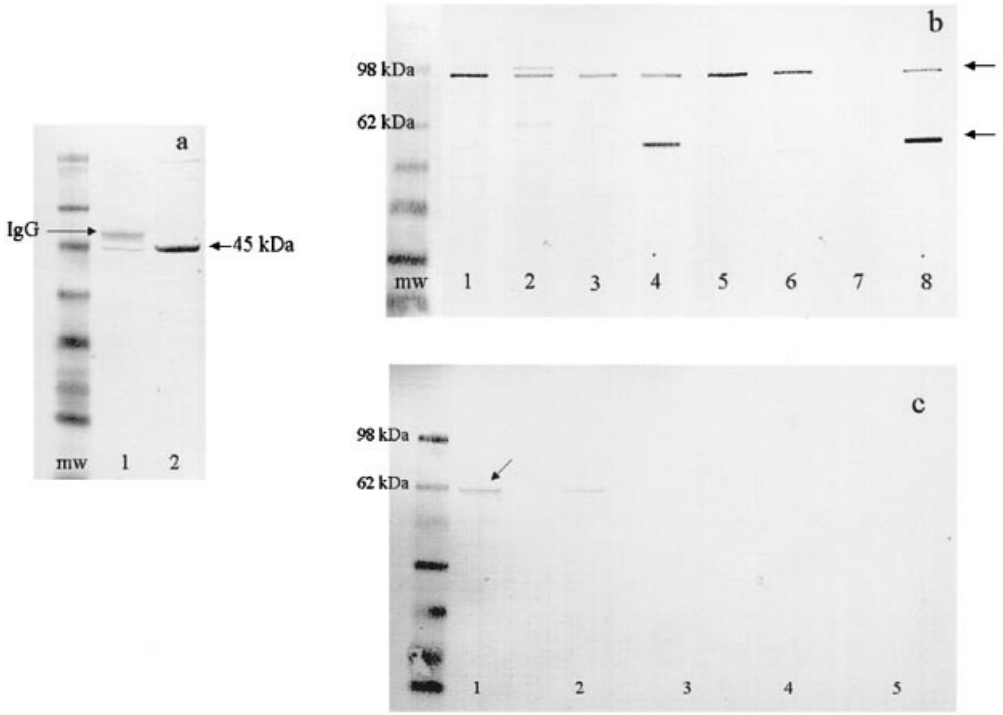

Fig. 2 (**a**) Autoantigens are common to human basal ganglia (lane 1) and rat brain (lane 2). The patient has autoantibodies against 45 kDa antigen, which is present in both human basal ganglia and rat brain. Secondary antibody (anti-human IgG) reacts with IgG in the human basal ganglia preparation only. (**b**) Rat brain western immunoblotting. Different patients (lanes 1–6 and 8) showed binding to a common 98 kDa antigen and a common 60 kDa antigen (lanes 4 and 8). Secondary antibody only (lane 7). (**c**) Regional localization of autoantigens in rat tissue. Patients with antibody reactivity against 60 kDa antigen incubated with the same protein concentrations of rat brain without cerebellum (lane 1), cerebellum (lane 2), kidney (lane 3), liver (lane 4) and heart (lane 5). The 60 kDa autoantigen is specific to (or enriched in) the brain.

Serum from different patients was incubated with rat brain immunoblots. The EL patients had antibodies against common autoantigens of molecular weight 40, 45, 60 and 98 kDa (Fig. 2b). The 45 kDa autoantigen often exists as a doublet.

We demonstrated the same anti-basal ganglia antibody binding in the CSF of four EL patients in whom the CSF was tested (Fig. 3). These patients with positive CSF anti-basal ganglia antibody had the following OCB isoelectric focusing

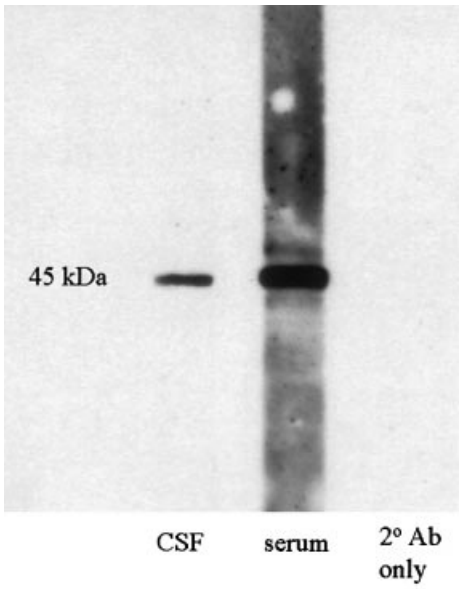

Fig. 3 Rat brain western immunoblotting. Patient with 45 kDa antibody binding. Comparison of CSF (diluted 1 : 5) with serum (diluted 1 : 2000). Antibodies against 45 kDa antigen are present in CSF as well as serum (polyclonal background noted in serum), confirming the presence of these antibodies in the CNS.

patterns: intrathecal OCB (n = 1), mirrored OCB (n = 2) and negative OCB (n = 1).

Regional localization of autoantigens in rat tissue

Using rat tissue, we compared the regional localization of the autoantigens (Fig. 2C). Western immunoblotting revealed that the 40, 45 (lower doublet), 60 and 98 kDa autoantigens were specific to or enriched in neural tissue. The higher molecular weight 45 kDa doublet was ubiquitous in all tissues. The 98 kDa autoantigen was also weakly expressed in heart. The autoantigens appeared to be equally present in both rat brain and rat cerebellum using these methods.

Immunohistochemistry (Fig. 4)

To localize the antibody binding, we performed immunohistochemistry on 10 EL patients who had positive western immunoblotting. All 10 had the same antibody binding pattern to axons and cell bodies of basal ganglia neurons (cytoplasmic binding), which were predominantly confined to tracts in the caudate, with less prominent binding in the putamen (Fig. 4). EL patients with western immunoblotting binding to the 40, 45, 60 and 98 kDa antigens all had similar immunohistochemistry findings. Ten control subjects from each control group had no binding to basal ganglia neurons.

We also examined two EL patients for antibody binding to midbrain neurons. Both had antibody binding to substantia nigra neurons, with similar neuronal staining to that seen in the caudate.

Staining of caudate/putamen with GFAP resulted in widespread staining of glial populations, as anticipated. The staining was ubiquitous throughout the slide (Fig. 5A). Unlike GFAP, patient IgG produced staining to tracts of cells that appeared to resemble neurons (Fig. 5B). Staining of sections using neuron-specific enolase revealed cytoplasmic cell body and axonal staining (Fig. 5C). Patient IgG produced a similar pattern of staining (Fig. 5D).

Histopathology

Case history

A 69-year-old male with a history of chronic obstructive airways disease presented following a febrile illness with profound somnolence and confusion. He became withdrawn and apathetic and stopped caring for himself. He subsequently developed bradykinesia and bilateral rigidity with cogwheeling. There were no pyramidal, cerebellar or sensory signs. Magnetic resonance neuroimaging revealed bilateral enhancement of the basal ganglia with associated swelling of the striatum and globus pallidus. CSF revealed no cells but intrathecal OCB. PCR of CSF for herpes simplex virus, varicella zoster virus and human herpes virus 6 and 7 was negative. His neurological syndrome progressed over 3 weeks and was complicated by a lower respiratory tract infection and subsequent respiratory failure, and the patient died.

Histopathological findings

Histological examination revealed perivascular lymphocytic cuffing, which was best developed in the basal ganglia but was also seen to a lesser extent in the cerebral cortex and cerebellum (Fig. 6A). Changes were most extensive in the putamen, globus pallidus and amygdala. There was no neuronophagia and no viral inclusions. Staining of striatal sections identified the infiltrating cell populations as T and B lymphocytes (Fig. 6B, C). Staining for amyloid and complement was negative. There were reactive astrocytes and activated macrophages in the striatal parenchyma.

Discussion

Over the last decade there has been increasing interest in immune-mediated movement and psychiatric disorders. The classic phenotype is Sydenham's chorea, which is characterized by chorea, motor weakness and behavioural disorders (particularly obsessive–compulsive disorder). The neurological syndrome occurs as a latent effect of group A streptococcal infections. More recently, motor tics in combination with a behavioural disorder have been described after streptococcal infections and have been termed paediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS) (Swedo *et al*., 1998). It

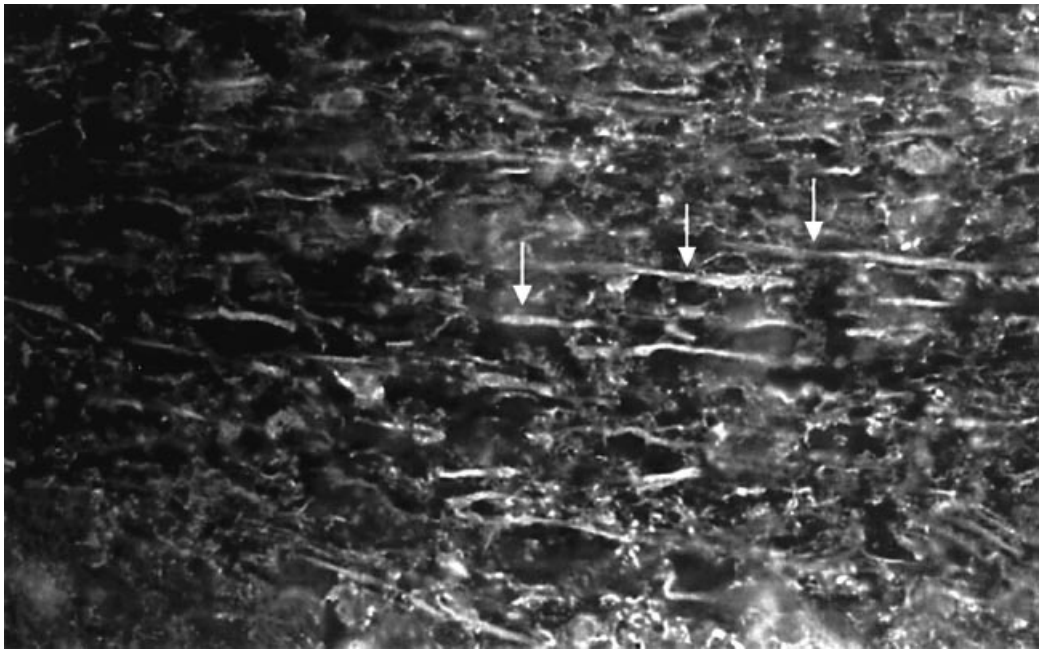

Fig. 4 Human basal ganglia immunofluorescence (arrows) in an EL patient, demonstrating antibody reactivity against tracts of basal ganglia neurons.

is hypothesized that antibodies induced after group A streptococcus infection cross-react with components of the basal ganglia, resulting in movement and psychiatric disorders. This hypothesis is supported by the presence of autoantibodies reactive against basal ganglia and subthalamic neurons in both Sydenham's chorea (Husby *et al*., 1976; Bronze and Dale, 1993; Church *et al*., 2002) and PANDAS (Kiessling *et al*., 1993). Recently, poststreptococcal dystonia as part of inflammatory autoimmune encephalitis has also been described (Dale *et al*., 2001). Although these descriptions suggest that the spectrum of poststreptococcal CNS disease is broader than previously described, the clinical phenotype remains predominantly localized to basal ganglia dysfunction (extrapyramidal movements and neuropsychiatric disease).

Recently, we have recognized a phenotype with remarkable similarity to EL. This mysterious disease was last seen in epidemic forms in the 1920s, and was described in detail by von Economo (von Economo, 1931). The central features of the disease were sleep disorder, lethargy, extrapyramidal movements (parkinsonism and dyskinesias) and neuropsychiatric disturbance (obsessive–compulsive disorder, catatonia, mutism, apathy and conduct disorders). The neuropsychiatric phenomenology of EL led to notions that the deep grey matter (particularly basal ganglia) may be involved in the control of mood, emotion, behaviour and volition (Cheyette and Cummings, 1995; Ward, 2003). Neuropsychiatric sequelae are now an accepted consequence of basal ganglia disease (Ring and Serra-Mestres, 2002). Furthermore, von Economo used EL as a model of sleep disturbance, and his anatomical localization of sleep control to the midbrain and deep grey matter has been confirmed by contemporary studies (Saper *et al*., 2001). The mortality of epidemic EL was between 20 and 40%. Of the survivors, many were left with parkinsonism, dyskinesias or psychiatric disease. The cases described in this series had similar characteristics to those described by von Economo. In this series, one of the patients died and five had a reduced consciousness level and required ventilation. It is probable that these ventilated patients would have died were it not for contemporary intensive care. Complete recovery occurred in a minority of patients in this series; the majority are still suffering continued psychiatric and movement disorders. The course of disease was monophasic or fluctuating, unlike the progressive course characteristic of the metabolic, biochemical or inherited causes of parkinsonism.

The CSF was abnormal in about 50% of epidemic EL cases, mild elevation of protein and mild lymphocytosis being characteristic (von Economo, 1931; McCall *et al*., 2001). The CSF abnormalities found in the patients in this report are similar to those in recent cases of sporadic EL (Howard and Lees, 1987; Blunt *et al*., 1997; Kiley and Esiri, 2001), and the presence of intrathecal synthesis of OCB has been proposed to be a useful marker of disease (Williams *et al*., 1979; Howard and Lees, 1987). Either intrathecal OCB or a mirrored pattern of OCB was seen in 69% of the patients described in this report. By contrast, all CSF PCR studies were negative, making a neurotropic viral encephalitis unlikely. When considered together, OCB, CSF pleocytosis and elevated CSF protein were abnormal in 81% of patients and, although in keeping with an immune-mediated pathogenesis, are not specific to this phenotype. The magnetic

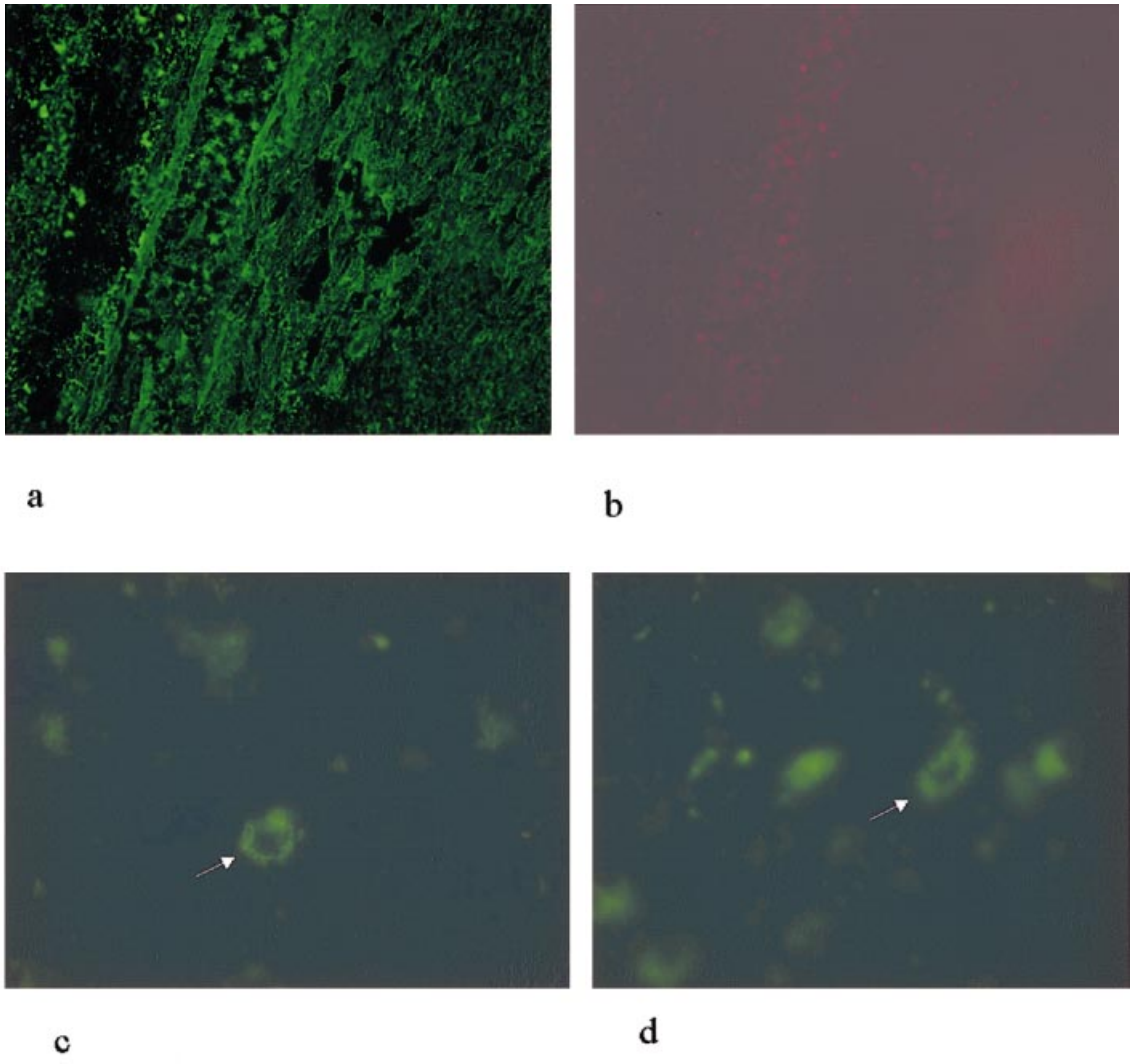

Fig. 5 (**a**) GFAP staining demonstrating widespread glial cell population staining with relative sparing of neuronal tracts, as anticipated. (**b**) Patient IgG demonstrating restricted localization of antibody binding to tracts of neurons, in contrast to glial populations. (**c**) Anti-neuron-specific enolase antibody demonstrating neuronal cell populations (cell body; arrow). (**d**) Patient IgG demonstrating antibody binding to neuronal cell populations with a similar staining pattern to neuron-specific enolase (cell body; arrow).

resonance neuroimaging in this cohort was abnormal in 40%; characteristic features were increased signal in the basal ganglia, substantia nigra and tegmentum, features recently proposed to be suggestive of EL (Verschueren and Crols, 2001). The enhancement resolved after the acute stage in the few patients who had convalescent imaging. Basal ganglia volumetric enlargement has been described in both Sydenham's chorea and PANDAS (Giedd *et al*., 1995, 2000). It would have been of interest to examine whether a similar phenomenon occurs in the EL patients who had normal conventional imaging.

The pathological features of both epidemic EL and contemporary EL have shown perivascular lymphocytic (plasma cell) cuffing, which predominantly involves the midbrain and basal ganglia (von Economo, 1931; Rail *et al*., 1981; Kiley and Esiri, 2001). A recent post-mortem case of a patient with the EL phenotype demonstrated an unexpected excess of perivascular plasma cells which were distended by IgG. The authors concluded that a brisk humoral response was occurring (Kiley and Esiri, 2001). We found similar histopathological findings with perivenous lymphocytic cuffing, predominantly of the basal ganglia. The lymphocytes were both T and mature B lymphocytes. Other than secondary reactive astrocytes and macrophage activation, there were no other striking pathological features. Of note, there were no viral inclusions. Interestingly, reports during the 1920s described remarkable pathological similarity between EL and Sydenham's chorea (Greenfield and Wolfsohn, 1922). Chronic changes included neuronal loss and neurofibrillary degeneration in the midbrain and basal ganglia.

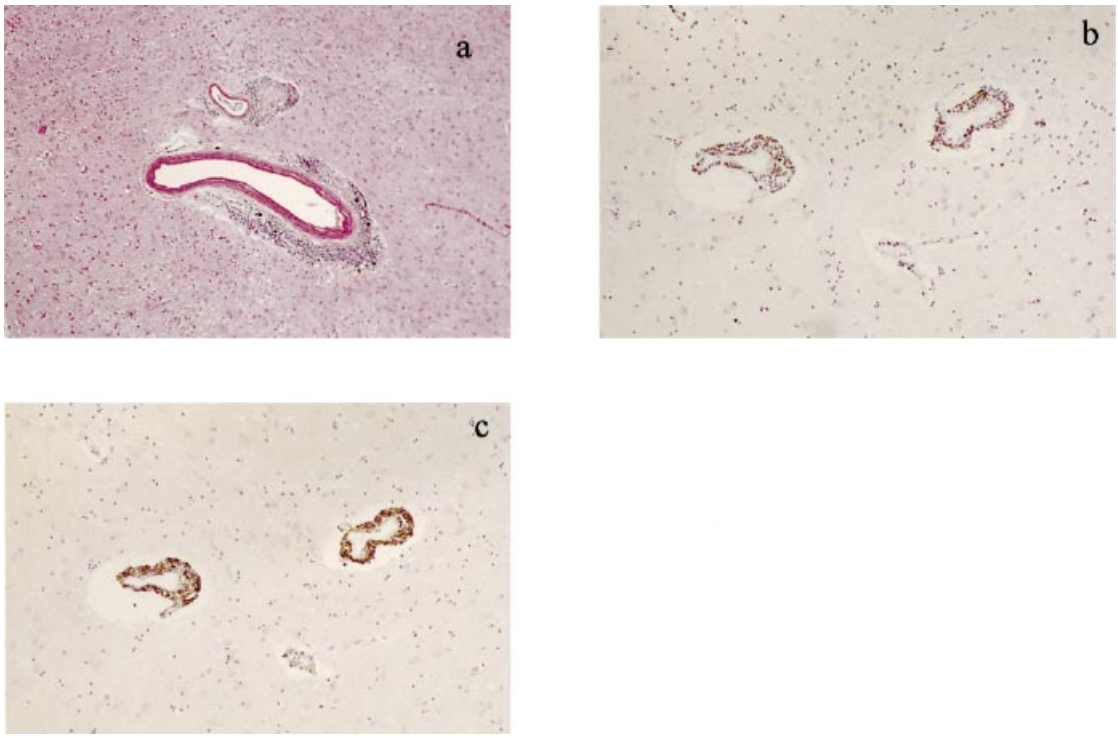

Fig. 6 (**a**) Histopathology of striatum demonstrates perivenous lymphocytic infiltration. Additional staining with (**b**) CD3 and (**c**) CD20 identifies infiltrating cells as T and mature B lymphocytes respectively.

The precipitating microorganism of epidemic EL remains unknown. Although EL has been associated with influenza (Ravenholt and Foege, 1982), von Economo suspected that an as-yet unidentified virus or alternative process was responsible. Studies during the 1920s showed no clusters of disease, suggesting an endemic presence as opposed to an imported epidemic (James, 1918). Unlike influenza, EL was not very contagious and even intrafamilial spread was unusual (von Economo, 1931). Further evidence against influenza being the cause of EL was that von Economo had observed EL for 3 years before the onset of the great influenza pandemic. In addition, patients with EL rarely had influenza before neurological onset; in one early report of 76 EL cases, only four had influenza in the preceding 6 months (Stallybrass, 1923).

Recent PCR examination of five acute EL brains from the 1920s epidemic revealed no influenza RNA. Unlike respiratory tissue from patients who died of the 1918 influenza viral pneumonia, brain tissue from EL patients had no influenza RNA. The authors concluded that the 1918 influenza virus was unlikely to have been directly responsible for the outbreak of EL (McCall *et al*., 2001). A separate recent study also demonstrated the lack of influenza genes in archived formalin-fixed brain samples of EL patients from 1916 to 1920 (Lo *et al*., 2003). Likewise, contemporary EL cases have failed to demonstrate evidence of invasive encephalitis secondary to neurotropic viruses (Duvoisin and Yahr, 1965; Williams *et al*., 1979; Rail *et al*., 1981; Howard and Lees, 1987; Elizan and Casals, 1989; Blunt *et al*., 1997; Kiley and Esiri, 2001), although one case occurred as a postinfectious complication of mycoplasma infection (Al-Mateen *et al*., 1988). A recent review concluded that, clinically, epidemiologically and morphologically, EL and the 1918 influenza virus represent distinct entities (Reid *et al*., 2001).

Interestingly, during the epidemic attention had focused on a streptococcus as a possible aetiological agent. A number of investigators had detected streptococci in the throats of patients suffering from EL (Harris, 1918; Wilson, 1918; von Economo, 1931). In addition, they were able to induce an EL-like illness in dogs after vaccination with streptococcal organisms (von Economo, 1931). However, as Gram-positive organisms were never isolated from the brain, the authors concluded that streptococcus was unlikely to have been the primary aetiological agent.

The hypothesis that the EL phenotype could be aetiologically similar to Sydenham's chorea is potentially appealing. Any hypothesis must be able to explain why the clinical, neuroimaging and pathological characteristics are relatively restricted to the deep grey matter. Invasive viral encephalitis rarely localizes so specifically. As pharyngitis was a common precedent of epidemic and contemporary EL, we examined the hypothesis that the EL patients described in this cohort were secondary to postinfectious autoimmunity directed against the deep grey matter. In this series, we were able to demonstrate recent streptococcal infection in significantly

more EL patients compared with neurological and healthy control groups. It is therefore possible that *Streptococcus* may play an aetiological role in this EL phenotype, as it does in SC. However a proportion of the EL patients in this cohort had no evidence of preceding streptococcal infection. We also acknowledge that serology is a potentially unreliable marker of previous infection, and growth of the organism would be preferable. However, autoimmune complications commonly occur many weeks or months after the precipitating organism, and growth of the organism is rarely possible. For example, in Sydenham's chorea the ASOT is elevated in only 73% of patients due to the latent onset of neurological disease after streptococcal infection (Taranta and Stollerman, 1956). We therefore propose that, although the association with streptococcus is a useful line of further investigation, alternative environmental triggers may be important in the disease evolution of this EL phenotype.

We were able to demonstrate autoantibodies reactive against discrete basal ganglia autoantigens in 95% of these patients. The low prevalence of anti-basal ganglia antibodies in streptococcal, neurological and dystonic controls suggests that anti-basal ganglia antibodies are unlikely to be due to an epiphenomenon or to be secondary to basal ganglia damage. Furthermore, rather than polyspecific binding, reactivity was seen against several common basal ganglia antigens. We believe that the 40, 45 and 60 kDa neural antigens are the important antigens in Sydenham's chorea (Church *et al*., 2002). These epitopes were also seen in the EL patients reported here, although a 98 kDa antigen was novel to this EL phenotype. Further investigation of these autoantigens showed them to be also present in rat brain. In addition, regional examination showed that the majority of the autoantigens are enriched in (or specific to) the brain, although we were unable to demonstrate a regional distribution within rat brain using these methods. Finally, CSF examination confirmed that the autoantibodies are present in the CNS, although whether these antibodies are mainly produced intrathecally or peripherally (with transfer into the CNS) has not been examined. It is acknowledged that the antigen preparation method used in this study is likely to have resulted in the presence of mainly soluble proteins, rather than lipophilic molecules such as membrane proteins (which would be the most likely targets for pathogenic autoantibodies). Ongoing proteomic studies will hopefully identify the autoantigens involved in antibody binding, and therefore their cellular localization and function.

Although we have demonstrated the presence of anti-basal ganglia antibodies in patients with an EL phenotype, we have not determined whether these antibodies have a functional effect on neurons and are therefore pathogenic antibodies. However, investigation of the PANDAS phenotype with an animal model (Hallett *et al*., 2000; Taylor *et al*., 2002) and a plasmapheresis treatment trial (Perlmutter *et al*., 1999) have suggested that anti-basal ganglia antibodies are pathogenic in these putative autoimmune phenotypes.

In conclusion, we believe that a phenotype with remarkable similarity to the historical descriptions of EL is still prevalent. As the present study was not epidemiological in design, but used a selected sample from tertiary referral centres, it is not possible to determine the prevalence of this disorder in the general population or to determine whether an epidemic of EL may return. In addition, we propose that this EL phenotype may occur secondary to postinfectious autoimmunity with apparent vulnerability of deep grey matter neurons. As there was no diagnostic test for EL during the 1920s, it is not possible to determine whether this contemporary disorder is an identical disease. However, we suggest that EL should once more be considered part of the neurological differential diagnosis. In von Economo's words, 'encephalitis lethargica can scarcely again be forgotten'.

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