

lent of that observed in culture, and consequently macrophages may be centrally involved in amyloid production. If that is correct, cells of the reticuloendothelial system may become targets for treatment in patients with myeloma.

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## Progressive multifocal leucoencephalopathy, sclerosing cholangitis, bronchiectasis and disseminated warts in a patient with primary combined immune deficiency

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### Abstract

**A 24 year old man presented with an unusual primary combined immune deficiency syndrome characterised by a profound lymphopenia of CD4 cells, selective serum IgG2 subclass deficiency, poor polysaccharide antibody responses, disseminated warts, recurrent sinopulmonary infection and bronchiectasis. The developed progressive multifocal leucoencephalopathy (PML) in association with sclerosing cholangitis.**

**Progressive multifocal leucoencephalopathy (PML) usually occurs as an opportunistic infection in patients with secondary defects in cellular immunity.**

The occurrence of progressive multifocal leucoencephalopathy (PML) as an opportunistic infection in patients with defective cellular immunity secondary to human immunodeficiency virus (HIV) infection, Hodgkin's disease, chronic leukaemia and immunosuppressive treatment is well known. Its association with primary immune deficiency is less well known and reports of its development in primary immune deficiency are exceedingly rare.<sup>1</sup>

### Case report

A caucasian boy born to unrelated parents in 1966 was first seen at another hospital at the age of 3 years on account of flexural eczema, recurrent tonsillitis, and otitis media. By the age of 5 a hoarse voice led to the detection and removal of vocal cord polyps. Two years later

generalised warts were first noted. In 1976 a right middle and lower lobe lobectomy was performed for bronchiectasis. Cystic fibrosis was excluded on the basis of a normal sweat test.

In December 1983 a dermatological opinion was sought for extensive warts which covered his neck, trunk, arms and knees. He was noted to be lymphopenic with a low T helper cell (CD4) count. Human papillomavirus type 3 was detected in a shave biopsy specimen of a wart by DNA hybridisation. He was given etretinate 20 mg daily for one year during which time there was some improvement that was not sustained. HIV serology was negative. In December 1984 Isoprinosine, a T cell stimulant was given (1 g four times a day) with no objective improvement in his warts. Isoprinosine was discontinued in July 1985. The lymphopenia of CD4 positive cells remained unchanged (fig 1). He was lost to follow up over the next five years until June 1990 when he was transferred to the neurology department at Oxford with a three month history of difficulty in walking, focal seizures, and jaundice of four weeks' duration.

On examination he was cachectic, dyspnoeic, and jaundiced. Disseminated warts and bilateral digital clubbing were noted. Neurologically he was partly orientated, followed simple commands, and could perform basic arithmetic. Bilateral optic atrophy, hypertonic limbs with ankle clonus, hyperreflexia, a right extensor plantar response and bilateral cerebellar deficits were noted. A cranial computed tomogram showed a left cerebellar hemispheric cyst with numerous low density lesions in both thalamic nuclei, right caudate

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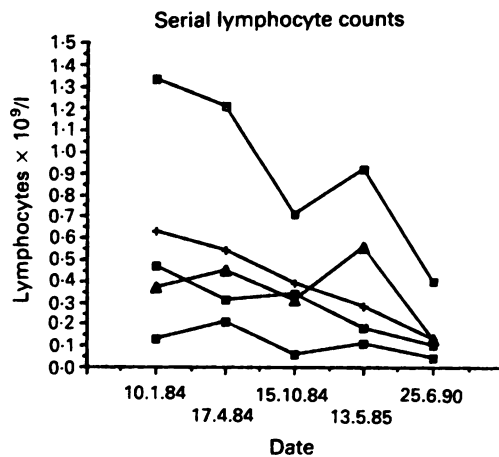
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Figure 1 Serial lymphocyte counts.



nucleus, and the right parietal lobe. A magnetic resonance imaging scan demonstrated high signal areas in both cerebral hemispheres, the basal ganglia and the left cerebellar hemisphere. Cell markers in peripheral blood are shown in fig 1. Delayed type hypersensitivity skin tests were precluded by the presence of extensive warts.

Table 1 Immunology

Serum	1972	1977	1984	May 1990	25.6.90
IgG (normal range 6–13 g/l)	9.7	17.3	13.1	12.0	10.4
IgG1 (normal range 3.2–10.29 g/l)					8.0
IgG2 (normal range 1.2–6.6 g/l)					0.64
IgG3 (normal range 0.2–1.9 g/l)					0.50
IgG4 (normal range 0.0–1.3 g/l)					0.54
IgA (normal range 0.8–2.5 g/l)	8.9	6.1	4.0	3.9	2.8
IgM (normal range 0.4–2.5 g/l)	0.5	0.38	0.14	0.5	0.51
IgE (normal range <125 U/l)	ND	ND	ND	ND	790
C3 (normal range 65–180 mg/dl)	ND	ND	ND	ND	158
C4 (normal range 15–50 mg/dl)	ND	ND	ND	ND	25.2
Functional antibodies:					
Tetanus	ND	ND	ND	ND	1/10 (low)
Diphtheria	ND	ND	ND	ND	1/32
<i>E coli</i>	ND	ND	ND	ND	1/512
Pneumococcal polysaccharide	ND	ND	ND	ND	< 1/16 (low)
Measles	ND	ND	ND	ND	< 1/8
Varicella zoster	ND	ND	ND	ND	1/16
Cytomegalovirus	ND	ND	ND	ND	< 1/8
Hepatitis B surface antigen					Neg
Antibodies to HIV			Neg	Neg	Neg
Syphilis serology				Neg	Neg
Cerebrospinal fluid					
Total protein	0.67 g/l (normal range 0.2–0.4)				
Lymphocytes	4				
Other cells	Nil				
Ziehl-Neelsen stain	Negative				
India ink stain	Negative				
Culture for acid fast bacilli	Negative				
Polyoma virus serology					
JC	Serum	GSF	Ratio		
BK	1:320	1:10	32:1		
Albumin (mg/dl)	1:1280	1:5	256:1		
Albumin (mg/dl)	3400	25	136:1		
IgG (mg/dl)	890	7.3	122:1		

ND = Not done

Table 2 Red cell purine and pyrimidine studies

Nucleotide ( $\mu\text{mol/l}$ )		Normal range
Adenosine triphosphate	2420	(1281 $\pm$ 123)
Adenosine diphosphate	178	(147 $\pm$ 38)
Adenosine monophosphate	16	(10 $\pm$ 4)
Guanosine triphosphate	92	(46 $\pm$ 10)
Guanosine diphosphate	17	(13 $\pm$ 3)
Nicotinamide adenine dinucleotide	104	(69 $\pm$ 11)
Nicotinamide adenine dinucleotide phosphate	63	(47 $\pm$ 8)
Uridine diphosphate glucose	69	(38 $\pm$ 8)
Orotidine	18	(normally undetectable)
Enzymes (nmol/mg haemoglobin/h)		
Adenine phosphoribosyl transferase	38	(16–32)
Hypoxanthine guanine phosphoribosyl transferase	143	(80–130)
Adenosine deaminase	78	(40–100)
Purine nucleoside phosphorylase	6490	(3000–7000)

There was a family history of optic atrophy affecting his maternal uncles, maternal grandmother, and three of her sisters and one brother. Analysis of his mitochondrial DNA showed a point mutation at a site different to that described by Wallace<sup>2</sup> in classic Leber's optic neuropathy (Poulton *et al*, personal communication). Further studies are in progress.

### Laboratory investigations

Results of immunological investigation are shown in table 1 and fig 1.

The results of chemical analyses are as follows: aspartate transaminase 99 IU/l (normal range 15–42); alkaline phosphatase 3379 IU/l (normal range 80–250);  $\gamma$ GT 357 IU/l (normal range 15–40); bilirubin 194  $\mu\text{mol/l}$  (normal range 3–17); calcium 2.09 mmol/l (normal range 2.1–2.6); phosphate 1.0 mmol/l (normal range 0.8–1.45); sodium 142 mmol/l (normal range 135–145); potassium 4.3 mmol/l (normal range 3.5–5.0); urea 4.1 mmol/l (normal range 2.5–6.7); creatinine 90 mmol/l (normal range 70–150); uric acid 164  $\mu\text{mol/l}$  (normal range 200–450);  $\beta$  2 microglobulin 2.5 mg/dl (normal range 0–3.0); caeruloplasmin 50.4 mg/dl (normal range 16–60).

The combination of a predominant defect in cellular immunity and hypouricaemia raised the question of purine nucleoside phosphorylase (PNP) deficiency. Detailed metabolic studies of the purine and pyrimidine pathways were undertaken in conjunction with Dr Anne Simmonds, Guy's Hospital, London, which excluded PNP deficiency (table 2).

A liver biopsy specimen showed focal portal tract fibrosis with a mixed inflammatory cell infiltrate, in the absence of ductular dilatation, highly suggestive of sclerosing cholangitis.

A diagnostic cerebellar biopsy specimen showed very large, bizarre, multinucleate astrocytes (confirmed by a positive reaction for glial fibrillary acidic protein and negative reactions for leucocyte common antigen and epithelial markers). Immunofluorescence studies performed at the Virus Reference Laboratory, Colindale, showed the presence of polyoma JC virus. DNA hybridisation using probes for human papillomavirus (HPV) 1, 2, 3, 4, 5, and 6 were negative. Following the diagnosis of PML active treatment was considered inappropriate and he gradually deteriorated and died a few days later.

A limited post mortem examination confined to the head showed a swollen brain (1505 g; forebrain 1328 g) with some autolysis and obvious softening of the left cerebellar hemisphere. The large vessels looked healthy while the leptomeninges were diffusely thickened. Acute haemorrhage was noted in the softened white matter of the right parietal lobe.

Histologically in all areas of the cerebrum there were multiple small and moderate sized lesions containing numerous enlarged oligodendrocyte nuclei with basophilic inclusion bodies typical of PML. Some lesions contained

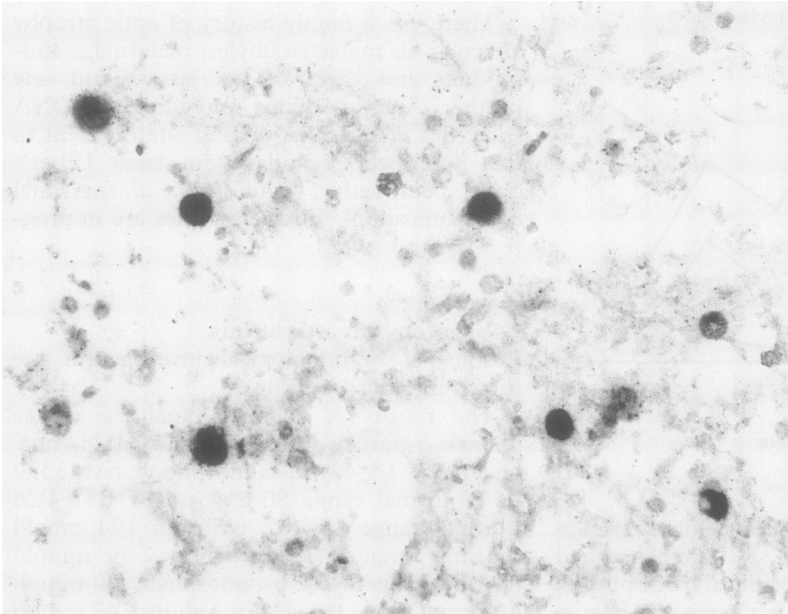


Figure 2 Cerebellar biopsy specimen hybridised with biotinylated JC virus specific nucleic acid probe. Dark nuclei of oligodendrocytes are those that contain virus (weak haematoxylin counterstain).

or were surrounded by astrocytes with bizarre enlarged nuclei as in the cerebellum. There was very little inflammation surrounding these areas except for increased numbers of reactive microglia. In situ DNA hybridisation for JC virus showed clear evidence of JC virus nucleic acid in many oligodendrocytes and in a minority of the bizarre astrocytes (fig 2).

### Discussion

The combination of a predominant cellular immune deficiency, intractable viral infection, a low serum uric acid and a progressive neurological syndrome were suggestive of PNP deficiency.<sup>3</sup> PNP is an important enzyme in the purine salvage pathway, deficiency of which leads to the accumulation of metabolites such as inosine, deoxyinosine, guanosine and deoxyguanosine which are toxic to lymphocytes (T>B). This results in impaired cellular immunity with relatively preserved humoral immune function in the early stages. With disease progression, however, substantial humoral immune deficiency is seen. Clinically, these patients present with a history of recurrent or persistent viral infection (particularly varicella zoster or cytomegalovirus) and often develop a spastic diplegia.

PNP deficiency was, however, excluded by the demonstration of normal levels of PNP activity in erythrocytes. The demonstration of an abnormal red cell nucleotide pattern (table 2) along with three unidentified pyrimidine metabolites is intriguing and raises the question of a hitherto uncharacterised error in pyrimidine metabolism.

The predisposition of immune deficient patients to develop extensive warts is well documented. Brooks *et al* have recently described a kindred with an X-linked combined immunodeficiency where five living males manifested severe varicella, chronic papilloma-

virus infection, and recurrent sino-pulmonary infection on a background of pronounced T cell lymphopenia, diminished lymphocyte proliferation to mitogens, decreased interleukin-2 production by mitogen stimulated lymphocytes and poor polysaccharide antibody responses, despite having normal total immunoglobulin concentrations.<sup>4</sup> Our patient's immune defect approximates closely to that described by Brooks. It would be interesting to see whether any of his patients go on to develop PML in the long term.

The recent description of primary combined immunodeficiency disorders due to defective expression of the T cell receptor complex (Ti-CD3) or impaired signal transduction in the presence of a normal Ti-CD3 complex has led to renewed interest in primary deficiencies of cellular immunity.<sup>5</sup> Patients with the severe form of T cell receptor immunodeficiency present as severe combined immunodeficiency (SCID), but patients with the mild form may remain asymptomatic or manifest a moderate combined immunodeficiency.<sup>5</sup>

Although our patient's lymphocytes expressed normal amounts of CD3 on their cell surface, the possibility of defective signal transduction cannot be ruled out because T cell functional studies were not carried out. To characterise these patients more completely it would be important to quantitate expression of the Ti-CD3 complex in addition to carrying out functional studies—delayed type hypersensitivity skin tests, lymphocyte transformation.

Despite clear evidence of impaired humoral immunity (low IgG2, poor polysaccharide antibody responses, bronchiectasis) in our patient it was felt that immunoglobulin replacement at this late stage would have been unlikely to influence the outcome. The benefits of immunoglobulin replacement in improving lung function have been proved in other antibody deficient patients. In contrast, immunoglobulin replacement is unlikely to have prevented or changed the clinical course of PML.

Serological surveys suggest that exposure to JC virus, the causative agent of PML, is fairly widespread. Although primary infection with the virus is not associated with an identifiable clinical syndrome, the virus may remain latent in the kidneys of some normal subjects until the onset of immunosuppression triggers reactivation. Mori *et al* have recently reported the detection of JC virus DNA in the brains of apparently immunocompetent elderly patients in the absence of PML, raising the possibility of viral latency in the brain as well.<sup>6</sup> Since its initial description in 1958 PML has proved to be remarkably resistant to treatment in most patients. A recent report which documents partial therapeutic success with the use of prolonged cytarabine treatment in HIV positive patients with PML is therefore encouraging.<sup>7</sup>

Glioma formation in immunosuppressed subjects with JC virus infection has been described.<sup>8</sup> Although the bizarre, large, multinucleated astrocytes which were present in the

biopsy specimens from our patient closely resemble those described in the case reported by Castaigne *et al*, we are not convinced that these cells were part of a truly neoplastic lesion in our case. There was a cyst present in one affected cerebellar hemisphere but no obvious tumour mass was detectable at surgery or necropsy. We therefore regard the bizarre astrocytes, a few of which clearly contained JC virus nucleic acid, as falling at the extreme end of the well recognised spectrum of pleomorphic astrocytic change seen in PML.

The cause of our patient's sclerosing cholangitis was unclear. Sclerosing and ascending bacterial cholangitis have been associated with campylobacter and cryptosporidial infection in patients with primary immune deficiencies.<sup>9</sup> It has been suggested that bacterial overgrowth in the upper small intestine may be an important predisposing factor in these cases. Interestingly, primary sclerosing cholangitis in immunocompetent subjects has recently been associated with significantly increased titres of serum antibodies to JC and or BK viruses.<sup>10</sup> This raises the possibility that reactivation of polyoma virus may have been responsible for

both PML and sclerosing cholangitis in our patient.

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## Isolation of *Legionella pneumophila* serogroup 14 from a human source

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### Abstract

**A strain of *Legionella pneumophila* serogroup 14 was isolated during a retrospective study, after death from the sputum of a patient who had had acute leukaemia and pneumonia. This is the third strain of that serogroup to be isolated from a human source. This event emphasises the importance of performing culture as well as serological tests, so as to detect cases of legionellosis caused by strains which rarely cause fatal clinical illness.**

Since 1976, when a "new" micro-organism, *Legionella pneumophila*, was first isolated,<sup>1</sup> 14 serogroups of this species have been described.<sup>2–7</sup> All serogroups are potential pathogens for humans, but some seem to cause illness more frequently than others.

To our knowledge, only two human isolates of *L pneumophila* serogroup 14 have been described<sup>2</sup>: one was isolated at the Minnesota Department of Health, Minneapolis, USA (strain 1169-MN-H); and the other was iso-

lated at Ruchill Hospital, Glasgow, Scotland (strain 1586-SCT-H).

### Case report

A 28 year old man in relapse of acute leukaemia after autologous bone marrow transplantation a year previously was admitted to hospital with a 10 day history of fever, followed by diarrhoea, epigastric pain, earache, and a white cell count of  $7 \times 10^8/l$ . A chest x-ray picture was unhelpful. Laboratory examinations showed antibodies to hepatitis viruses A and B. The patient was initially treated with imipenem, and thereafter with teicoplanin and ceftazidime. After a positive blood culture for *Candida pseudotropicalis* vancomycin and amphotericin were added. He developed a scantily productive cough. Ceftazidime was replaced with piperacillin. A chest x-ray picture four days later showed the presence of confluent densities in the middle right and lower lobes and the left parahilar area. Antibiotic treatment was changed to imipenem and cotrimoxazole. Dyspnoea and peripheral cyanosis appeared the following day, and the

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