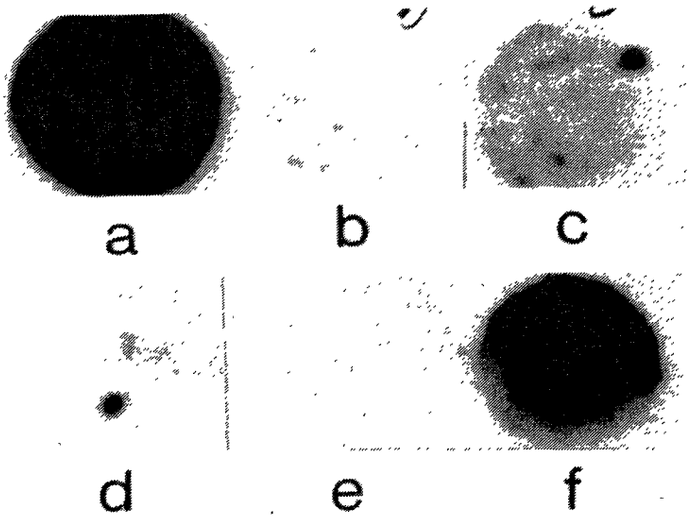

HPV-FISH autoradiographs on DNA.

(a) 3+, (c) 2+, (b) and (d) 1+, oesophageal carcinomas; (e) negative oesophageal carcinoma; (f) hela cell line containing HPV-18 DNA sequences[9] and used as positive control.

the carcinomas and of adjacent non-neoplastic oesophageal epithelium were examined histologically. Binucleation and/or perinuclear clearing similar to koilocytosis was noted in epithelium adjacent to carcinoma tissue in about 25% of the cases. However, distinctive changes identifiable as definite HPV effect were not seen. The complex of appearances attributable to HPV in the cervix—koilocytosis with nuclear atypia, multinucleation, papillomatosis, parakeratosis, and basal cell hyperplasia—was not present in any case. On examination of the tissue for genus-specific HPV capsid antigen by the avidin-biotin immunoperoxidase method (Vector Laboratories, Burlingame, California) no case was positive, either in the tumour or in adjacent squamous epithelium.

DNA hybridisation studies were done in 10 cases of oesophageal carcinoma using a method developed for paraffin-embedded material, the accuracy being verified by one of us (J. K.) using tissue and smears of cervical HPV infections. The method involves a combination of extraction of nuclei from tissue[7] and filter in-situ hybridisation (FISH).[8] Hybridisation was done under stringent conditions, using a mixed probe of HPV types 11, 13, 16, and 18 obtained from Dr H. zur Hausen and Dr L. Gissmann (Deutsche KrebsforschungsZentrum, Heidelberg, West Germany). In 5 cases oesophageal carcinoma tissue was positive. 1 case was graded 3+ positive (figure, a), 1 as 2+ positive (c), and 2 cases showed only minor positivity (b and d).

The DNA hybridisation method used here is being further evaluated for sensitivity and specificity. However, from these preliminary observations it seems that HPV DNA is present in some oesophageal carcinomas in a low-risk area and that HPV is worthy of further investigation as a possible aetiological factor in oesophageal carcinoma. Hybridisation of DNA extracted from paraffin-embedded material could be applied to archival tissue from various body sites, offering a simple screening test for the detection of viral DNA and, perhaps, other DNA in tissues.

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DR2-NEGATIVE NARCOLEPSY

SIR,—A recent addition to the long list of HLA-associated diseases is narcolepsy (with DR2).[1-3] The association seems strong, DR2 being found in 100% of cases with narcolepsy in some series. However, in a study of 118 such patients (21 from Munich, 97 from Prague) we found 4 who lacked DR2 on duplicate HLA DR/DQ serotyping with confirmatory tests with gene probes for DR and DQ β chains.

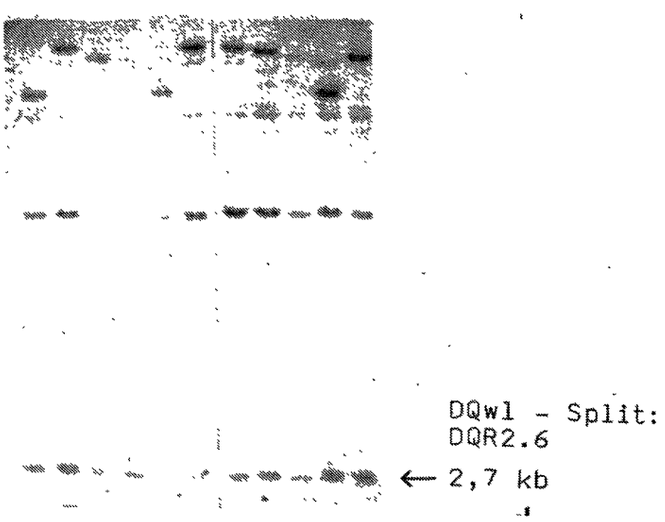
Rigorous clinical evaluation included sleep laboratory studies in most patients and only patients with an unequivocal diagnosis of narcolepsy were included.

The 4 DR2 negative narcolepsy patients possess either DR4 or DR5 or both, and all are positive for DQw3 (table). In 4 other published DR2-negative cases[4-6] DR types are provided for 3 (DR1/DR4, DR1/DR3, DR4/DR7).

HLA PHENOTYPES OF DR2-NEGATIVE NARCOLEPSY PATIENTS

Patient	HLA					DQR2·6 band	Cataplexy
	A	B	DR	DRw	DQ		
1	11, 24	55	4, 5	52, 53	3	−	+
2	2	60, 61	1, 5	52	1, 3	−	+
3	2	35, 49	4, 5	52, 53	3	−	−
4	2	60, 62	4, 6	52, 53	1, 3	+	−

2 of our 4 patients had the full picture of narcolepsy, including cataplexy, and cannot be distinguished clinically from DR2-positive cases. However, the other 2 did not have cataplexy. 6 out of 6 additional patients from Prague with narcolepsy after organic brain damage (encephalitis or brain trauma) were positive for DR2 and all had cataplexy. Perhaps a genetic predisposition to narcolepsy existed before the organic brain damage, which may or may not have contributed to the onset of narcolepsy.



***Eco*RI digest of genomic DNA from narcolepsy patients, hybridised with a DQ-β probe (P. Peterson).**

10 patients DR2 positive, 1 patient (patient 1) DR2 negative (arrow).

We have not identified any DR-β polymorphism which correlates with a subtype of DR2 in narcolepsy. However, with an *Eco*RI digest of genomic DNA and a DQ-β probe we have identified a 2·7 kb band, correlating with DR2 and DRw13 (a subtype of DRw6),[7] which is almost certainly identical to DQR2·6[8] (figure). We have found this DQR2·6 band in all 27 DR2-positive patients tested at the DNA level so far, which confirms the data of Marcadet et al.[8] In the DR2-negative patients, however, we do not find this band (figure), except in the patient with DR4, DRw6, where it would be expected (on the DRw6 haplotype). In DR2-positive healthy controls we found the DQR2·6 band in 19 of 21 cases—ie, much more frequently than in Marcadet's controls. However, our DR2-positive healthy panel contained a high proportion of DR2 individuals who carried HLA-B7 (11 of 21), a combination strongly associated with DQR2·6.

We conclude that narcolepsy cannot be excluded just because the patient is negative for DR2 and that the DQR2·6 subtype of DQw1, which has been found to be highly associated with narcolepsy, is negative in the DR2-negative patients. It remains to be seen whether DR2-negative narcolepsy patients have any HLA antigens in common.

We thank Prof Per Peterson, Uppsala, for the DRβ and DQβ probes; Dr D. Cohen, Ms M.-P. Font, Dr E. Weiss, and Prof H. Wolf, Munich, for their help in the establishment of the technique; and Ms Annette Grooms for technical assistance. Rabbit complement was donated by Behring-Werke, Munich. Supported by DFG Sonderforschungsbereich 217, project A2.

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SPIROCHAETES, LYME DISEASE, AND MULTIPLE SCLEROSIS

SIR,—Dr Muhlemann and colleagues (May 10, p 1097) and Dr Fumarola (Sept 6, p 575) state that we have proposed an association between multiple sclerosis (MS) and *Borrelia burgdorferi*, the causative organism of Lyme disease. This is not so. Lyme disease, being exclusively a tick-borne infection with characteristic epidemiological features (localised epidemics, restricted to persons exposed to specific ticks and occurring in the summer months) is wholly inconsistent with the epidemiology of MS. Moreover, Muhlemann et al have found no serological evidence to support *B burgdorferi*, or any other closely related *Borrelia* sp, as a possible cause of MS.

What we did note (April 12, p 819) is that spirochaetal infections, such as Lyme disease, have many of the "immunological and pathological characteristics of MS", suggesting the possible spirochaetal aetiology of that disease. Indeed we think that MS may be caused by a treponemal spirochaete that invades the central nervous system through defects in the wall of the sphenoidal sinus (July 12, p 75), and we have evidence from immunofluorescence and western blot studies that an organism of this type is present in the central nervous system in active MS (unpublished).

We should point out, however, that there is now clear evidence that *B burgdorferi* may occasionally cause demyelination as a sequel to Lyme disease.[1,2] It seems inevitable that some patients, especially in areas where Lyme disease is endemic, who have been labelled as "possible MS", will ultimately be shown to have Lyme demyelinating encephalopathy. A search for these patients should be undertaken in endemic areas.

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AMINOACID LOSSES ON HAEMOFILTRATION

SIR,—Intravenous aminoacid solutions are often given to patients with acute renal failure who cannot be fed enterally. Aminoacid losses on conventional haemodialysis are small[1] but little is known about losses during haemofiltration because the molecular weight cut-off for the filter (30 000 or more) is much greater than the molecular weights of individual aminoacids.

Continuous veno-venous haemofiltration (CVVHF) was used in the treatment of a 29-year-old woman in acute renal failure due to Goodpasture's syndrome and who was also unconscious due to vasogenic cerebral oedema. 24 litres of haemofiltrate were collected daily for 7 days via a Gambro FHSS haemofilter, the rate then being reduced to 12 litres per day for a further 7 days. During this period she received no oral feeding, remained anuric, and passed no faeces. She was parenterally fed, whilst on CVVHF, with 500 ml of 50% dextrose with insulin and added vitamins, 1 litre of 'Vamin 9' by continuous infusion over 24 h, and 250 ml of 20% 'Intralipid' each day. Haemofiltrates were collected for each 24 hour period with a corresponding timed serum sample and assayed for aminoacid content ('Chromaspek'; Rank Hilger, Margate). Samples were analysed daily for each week at the two different filtration rates, and the results are expressed as mean (and standard error) for 7 days. A

SERUM AMINOACID PROFILES AND CLEARANCES IN PATIENT ON HAEMOFILTRATION AT RATES OF 24 (A) AND 12 (B) LITRES DAILY: MEAN (AND STANDARD ERROR)

	Serum (μmol/l)		Clearance (ml/min)	
	A	B	A	B
Ala	409·2 (53·9)	437·8 (42·6)	31·1‡ (1·34)	4·6 (0·716)
Glu	426·3 (54·6)	327 (29·7)	12·5* (4·49)	4·2 (0·845)
Val	328 3 (67·6)	182·8 (31·4)	9·9† (1·57)	7·6 (1·52)
Leu	178·7 (38·8)	127·8 (16)	7·5 (1·26)	5·6 (1·10)
Iso	78·7 (16·5)	72·3 (10·9)	10·6 (2·0)	6·5 (1·11)
Tyr	48·8 (24·4)	62·5 (16)	10·9 (2·21)	7·2 (2·18)
Phe	210·5 (35·5)	174·2 (26·2)	13·9‡ (1·37)	5·3 (1·25)
Cys	115·5 (23·4)	96 (17·4)	10·2 (0·803)	11·7 (3·73)
Met	21·8 (5·35)	23·5 (2·63)	11·2‡ (3·02)	6·3 (2·03)
Gly	409·7 (53·3)	397·3 (49·7)	14·4* (0·956)	5·8 (1·08)
Pro	314 (34 7)	260·3 (42)	15·4* (1·74)	8·7 (2·19)
Thr	231·8 (83·6)	136·3 (24·8)	7·8* (0·878)	5·2 (0·733)
Ser	191·5 (36·2)	158·3 (17·7)	7 8‡ (0·293)	4·5 (0·24)
His	94·2 (34·3)	72·3 (15·3)	22 (7·26)	14·1 (5·08)
Asp	67 (15·4)	44 5 (7·03)	7·4 (0·667)	6·1 (1·32)

Creatinine clearances were 25·5 ml/min (SE 1·56) for 24 litres daily and 8 08 ml/min (SE 0·48) for 12 litres daily.
*$p < 0·05$, †$p < 0·01$, ‡$p < 0·001$; otherwise differences were not significant.