deficiency and reflects the importance of this serine protease inhibitor for the anticoagulant activity of heparin.

Our experience shows the importance of screening for an underlying disorder in the young with thrombosis and strongly suggests that prophylactic measures aimed at preventing thrombosis should be considered in acutely ill subjects with infection and familial thrombophilia.

No evidence of HTLV-I infection in French patients with multiple sclerosis using the polymerase chain reaction

E Wattel, M Mariotti, J D Bignon, F Agis, E Gordien, J Y Muller, J Hors, J J Lefrère

Abstract
The polymerase chain reaction (PCR), using three primer pairs in the pol, tax, and env regions of the HTLV-I genome, was unable to detect HTLV-I in the blood samples of 54 caucasian subjects with multiple sclerosis who were seronegative for HTLV-I/II. Seventeen HTLV-I/II seropositive (by ELISA and Western blot) subjects used as positive controls were positive with the three primer pairs. The PCR was negative in 47 healthy HTLV-I/II seronegative (by ELISA) subjects at low risk of HTLV-I infection used as negative controls.

These results suggest that there is no association between the occurrence of HTLV-I sequences and the development of multiple sclerosis.

As these results have not been confirmed by others, however,3,4 Reddy suggested that these discrepancies could have been due to the fact that only a subgroup of such cases could harbour HTLV-I sequences.5

Doubts therefore persist as to the possible relation between HTLV-I infection and MS. We decided to study a large number of French patients with MS to see if HTLV-I sequences could be detected using a PCR assay, to check if epidemiological differences could explain the discrepancies in previously published assays.

Methods
Fifty four caucasian adults with MS, as defined by Poser et al7, were studied. Mean age at onset of disease was 29.4 years; mean age at the time of the study was 42.2 years; 17 were male. All were seronegative by ELISA for antibodies to HTLV-I/II. None had a history of blood transfusions.

Seventeen HTLV-I/II seropositive (by ELISA) subjects living in an endemic region of HTLV-I/II infection (Guadeloupe, French West Indies) and showing typical patterns of HTLV-I/II infection on Western blotting were used as positive controls. Forty seven healthy HTLV-I/II seronegative (by ELISA) subjects (blood donors), living in a non-endemic area (Paris), and with no risk factors for HTLV-I/II infection were used as negative controls.

DNA EXTRACTION
DNA was prepared from 5 ml of fresh or
frozen peripheral blood. Red cells were lysed by a hypotonic buffer (TRIS-HCl 10 mmol/l, pH = 8.6; magnesium chloride 10 mmol/l; sodium chloride 10 mmol/l). The resulting pellet was washed with the same buffer. DNA was extracted after treatment of the cells in lysis buffer (TRIS-HCl 10 mmol/l, pH = 8.6; EDTA 10 mmol/l, pH = 8; sodium chloride 10 mmol/l; sodium dodecyl sulphate 0.5%; protease K 100 μg/ml) at 42°C overnight. After phenol and chloroform isoamyl alcohol (1 in 24) treatment the DNA was precipitated with ethanol and resuspended in a solution of TRIS-HCl (10 mmol/l, pH = 7.5) and EDTA (1 mmol/l).

**PRIMERS AND PROBES**
The primers used for PCR amplification and the probes used to detect amplified sequences were derived from the pol, tax, and env regions of HTLV-I sequences: (i) The two pol primers were SK54 and SK55 and would be expected to amplify the region between nucleotides (nt) 3365 to 3483 of the sequence described by Seiki.

Our oligonucleotide probe was SK36, which spans the region between nt 3426 and 3460. (ii) The two env primers have been previously described and would be expected to amplify the region between nt 5684 to 6151 of the HTLV-I sequence. The oligonucleotide probe was SG 228, which spans the region between nt 5841 and 5880. (iii) The two tax primers were SK43 and SK44 and would be expected to amplify the region between nt 7358 to 7516 of the HTLV-I sequence. The oligonucleotide probe was SK45, which spans the region between nt 7447 and 7486 of HTLV-I sequence.

**PCR METHODOLOGY**
Amplification of specific fragments by PCR with thermostable Taq DNA polymerase was carried out as follows: 100 ml reaction volumes contained 1 μg genomic DNA in 10 mM TRIS-HCl (pH = 8.3), 2.5 mM MgCl2, 200 μM of each dNTP (dATP, dCTP, dGTP, dTTP), 0.2 μmol/l of each primer and 2.5 units of enzyme. The samples were overlaid with several drops of mineral oil and subjected to 35 cycles of amplification: DNA denaturation was carried out at 94°C for one minute; annealing of primers was carried out at 59°C for one and a half minutes; extension of the two primers was carried out at 72°C for one and a half minutes. To prevent contamination, negative controls were included in each run, and a control reaction looking for a single copy cellular gene—for example, β globin was performed with each sample to ensure that the DNA was amplifiable. We tested our strategy with an MT-4 cell line infected with HTLV-I. Analysis of amplification products was studied through slot blotting and Southern blotting (from a 2% agarose gel). The gel was stained with ethidium bromide and photographed under ultraviolet light, showing a specific band.

**RESULTS**
Patients with MS did not have evidence of HTLV-I sequences with the three primer pairs used. The 17 HTLV-I/II seropositive subjects were all positive for HTLV-I with primer pairs in pol, tax, and env regions. The polymerase chain reaction assay was negative with the same primer pairs in the 47 healthy HTLV-I/II seronegative subjects who acted as negative controls.

**DISCUSSION**
In the controls amplification was specific for HTLV-I sequences and no amplification of non-specific sequences occurred. Our results in patients with MS conflict with those of Reddy and Greenberg, and confirm those of Bangham and Richardson. These discrepancies are difficult to explain. As some cases of tropical spastic paraparesis for which the association with HTLV-I infection has been confirmed can clinically resemble multiple sclerosis, HTLV-I positivity in MS might have been tropical spastic paraparesis. Furthermore, the exquisite sensitivity of the PCR assay is such that DNA contamination from the laboratory can lead to false positive results. In Reddy’s study one of the 20 negative controls showed detectable HTLV-I sequences.

Our data strongly suggest that an association between the occurrence of HTLV-I sequences and the development of multiple sclerosis is unlikely. Nothing suggests that this retrovirus may be responsible for MS. We conclude that the discrepancies of results suggest that a quality control for laboratories using the PCR in HTLV-I is indispensable.

We thank L. Prin for the MT4 cell line infected with HTLV-I, and AM Courouce and O. Prou for HTLV-I serology (Western blotting). We also thank Annick Alperovitch and the French research group on multiple sclerosis for providing the blood samples from patients.