will be necessary to address these issues and may improve our understanding of the immunology of organ transplantation.

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A urinary marker for multiple sclerosis

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A toxic factor for macroglial cells has been described in monocyte cultures in vitro and in cerebrospinal fluid (CSF) from multiple sclerosis (MS) patients, as reported in the first communication.[1] This gliotoxic activity is a form of apoptosis and can be detected by a diethyl-tetrazolium salt-based in-vitro assay. Having established a flow-cytometry procedure to detect staurosporine-induced apoptosis in immortalised glial cells, we have applied this method to optimisation of gliotoxic activity detection in body fluids.* This gliotoxic activity was related to a protein of molecular weight 17 kDa. Since other data indicated passage from CSF to blood, we supposed that glomerular passage of this protein was also possible. We identified gliotoxic activity associated with a protein with identical biochemical characterstics to that in urine from patients with MS.

We studied urine samples because they are easy to obtain and allow extensive and repeated sampling, which is not practical or ethical with CSF. Total urine (50 μl per well) was heated at 56°C for 30 min, passed through 0·2 μm filter, directly added to cell-culture wells, and incubated for 72 h. After DNA strand-break extraction and propidium iodide staining, apoptosis was measured by comparison with normal proliferating cells. We report our initial findings of gliotoxic activity in urine from 104 individuals. For patients in hospital, urine was collected immediately on admission before the start of treatment. Group 1 included 35 patients with definite MS according to Poser's criteria.[2] The clinical course and status were described with the EDMUS impairment scale.[3] Group 2 included 34 patients with other neurological disorders: 25 with central nervous system illnesses, eight with peripheral nervous system disease, including inflammatory neuropathies, and one psychiatric patient. Group 3 consisted of 35 healthy volunteers. Urinary gliotoxic activity was tested twice independently in all individuals.

The results are shown in the table. 32 of 35 patients with MS had a positive test—ie, glial cell apoptosis induced by urine. The three cases with negative findings had a disease duration of less than 10 years, with an attack at the time of sampling but a low disability score. Nevertheless, these characteristics did not suggest a particular clinical profile, since they were also found in two other MS patients with a positive test. All healthy controls had a negative test as did 33 of 34 patients with other neurological diagnoses. The only exception was a patient with benign fasciculations.

	MS urine	Non-MS urine	
	Mulltiple sclerosis (n=35)	Other neurological disorders (n=34)	Healthy controls (n=35)
Apoptosis	32/35 (91%)	1/34 (3%)	0/35 (0%)
No apoptosis	3/35 (9%)	33/34 (97%)	35/35 (100%)

Detection of apoptosis induced on an immortalised macroglial cell line by urine samples from MS patients, non-MS patients, and healthy controls

Our results show a strikingly high sensitivity (91%) and specificity (97%) of this urinary marker in MS. It is worth noting the negative results in patients with other neurological diseases, including Guillain-Barré syndrome, centropontine myelinolysis, and brain glioblastoma. During the past 20 years, several investigators have failed to identify a suitable biological marker of multiple sclerosis, including myelin basic protein.[4] We demonstrate here that this gliotoxic factor can simply and repetitively be detected without invasive sampling. Our findings may be useful in the diagnosis or the follow-up of MS, and may help to assess therapeutic strategies. It might be interesting to correlate urine gliotoxic activity with magnetic-resonance-imaging-detected brain and spinal cord lesions, as well as with the intensity of relapses. The characterisation of the peptide sequence of this gliotoxic factor, which may be a glycoprotein, is now a priority.

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*Detailed protocol available from the authors, on request.

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HSV-1 in brain and risk of Alzheimer's disease

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We have shown that the incidence of herpes simplex virus 1 (HSV-1) in the brain is similar in elderly individuals with and without Alzheimer's disease (AD).[1] We have also shown that individuals carrying the ε4 allele of apolipoprotein E (apoE) are at increased risk of developing AD.[2] Itzhaki and colleagues[3] have shown that the combination of HSV-1 in the brain and presence of the ε4 allele of apoE together confer an even greater risk for AD when compared with individuals carrying the ε4 allele alone.[3]

We investigated brains from 73 elderly individuals with neuropathologically confirmed AD (34 men, mean age 77·2 [range 53–93] years) and 33 without AD (27 men, mean age 72·2 (43–95] years) based on criteria established by