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Letter

Elevated levels of serum S100 beta protein in scrapie hamsters

Recently, immunoluminometric analysis revealed elevated levels of serum S100 beta protein in sporadic and genetic cases of Creutzfeldt-Jakob disease (CJD) as compared to demented and non-demented control patients (Otto *et al*, 1998). S100 beta protein is a well established marker for the activation of astrocytes and also shows enhanced histochemical presentation in Alzheimer's disease (Sheng *et al*, 1997). Thus, it will be necessary to precisely assess the specificity of the new test for human transmissible spongiform encephalopathies (TSE) in larger study populations.

Notwithstanding the need for further case-control studies, the high homology of S100 beta protein in man and cattle (Jensen *et al*, 1985) suggests that the protein might be used for the diagnosis of bovine spongiform encephalopathy in clinically inconspicuous animals as a protective measure for human health. This requires to first establish whether abnormal concentrations of S100 beta protein can also be found in infected individuals, either in animals exposed to the transmissible agent or in humans suffering from new variant Creutzfeldt-Jakob disease (vCJD), a form of CJD most likely originating from bovine spongiform encephalopathy (BSE) (Bruce *et al*, 1997, Hill *et al*, 1997). Therefore, we investigated serum levels of S100 beta protein in scrapie-infected hamsters, a well-established animal model for transmissible spongiform encephalopathies.

The analysis was performed in 36 Syrian golden hamsters having reached the terminal stage of disease after intracerebral or intraperitoneal infection (18 animals each) with scrapie strain 263K, and in 23 non-infected controls. All hamsters for the i.c.-series were taken from bioassay experiments. These recipients had been inoculated with various amounts of infectivity and showed incubation times in the range of 83–146 days. The i.p.-infected animals received a uniform dose of agent ($2*10^7$ LD_{50}) and developed scrapie at 111 ± 11 days post infection (d.p.i.). For euthanasia animals were exposed to CO_2 and 2 ml of blood were removed by puncture of the right cardiac ventricle immediately after cessation of breathing. The blood was incubated for 15 min at 37°C and centrifuged for 10 min at 10000 r.p.m. (Eppendorf centrifuge). The serum was separated from the blood clot and subsequently kept frozen until analysis. In order to prevent an artefactual increase in S100 beta protein concentration (data not shown) isolation and freezing of the serum was accomplished within 3 h after blood collection.

The determination of serum S100 beta protein levels in blinded samples from scrapie-infected and normal hamsters according to the previously published method (Otto *et al*, 1998) revealed a significant difference between the infected and the control group ($P < 0.0001$; Mann-Whitney U-test). There was also a difference between i.p. and i.c. infected animals, although on a lower level of significance ($P < 0.05$; Mann-Whitney U-test). As shown in Figure 1 hamsters with terminal scrapie exhibited elevated levels of serum S100 beta protein in the range of 78–1021 pg/ml (median: 226 pg/ml; mean: 304 pg/ml) after i.c.- and 23–451 pg/ml (median: 130 pg/ml; mean: 164 pg/ml) after i.p.-infection. Only 10–107 pg/ml (median: 20 pg/ml; mean: 32 pg/ml) were found in the non-infected animals.

The previous case-control study in humans revealed about fourfold higher median serum levels of S100 beta protein in CJD cases (median: 395 pg/ml; mean: 387 pg/ml) than in patients with non-TSE diseases (median: 109 pg/ml; mean: 177 pg/ml) (Otto *et al*, 1998) . The median serum concentrations of the protein in i.c.- and i.p.-infected scrapie hamsters were considerably lower, but corre-

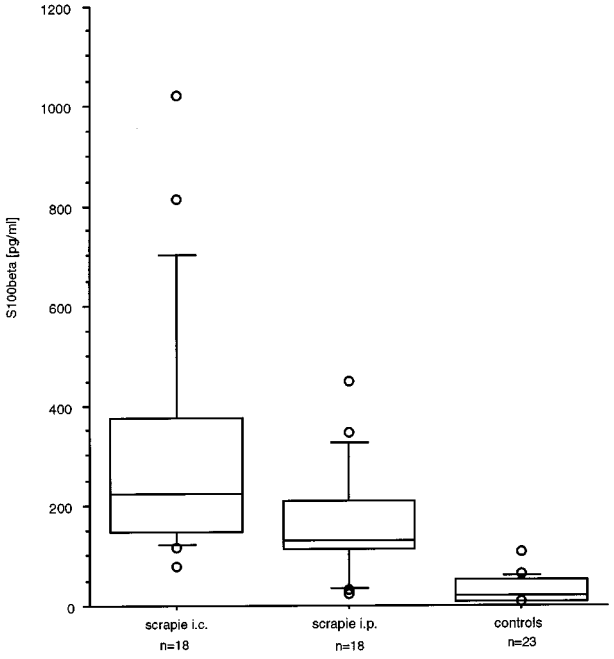

Figure 1 Serum levels of S100 beta protein in scrapie (i.c., intracerebral infection; i.p., intraperitoneal infection) and control hamsters. Boxplot shows 10th, 25th, 50th, 75th, 90th percentiles and outliers

sponded to a higher increase of about 11- and 6.5-fold, respectively, as compared to the baseline found in the controls. Whether these differences are related to the host species or to the strain of agent, remains to be established.

For the i.c. as well as for the i.p. group we observed the occurrence of high and low outliers. These animals were neither consistently related to particularly long or short incubation times nor did they show significant differences in their incubation periods. Despite the outliers, a cut-off level of 65 pg/ml allowed the diagnosis of scrapie with 100% sensitivity in i.c.- and 83% sensitivity in i.p.-infected animals. For both routes of infection the specificity of the test was 96%.

Our findings provide first evidence that increased concentrations of serum S100 beta protein are not restricted to sporadic or genetic CJD but are also associated with transmitted disease and TSEs in animals. This suggests that elevated levels of the protein may also be found in cattle with BSE and in patients with vCJD. In some rodent CJD models, astrocyte activation has been observed about 150 days prior to the manifestation of clinical symptoms (Manuelidis *et al*, 1997). Current kinetic studies on the serum and cerebrospinal fluid of intraperitoneally and orally infected hamsters will reveal at which stage of incubation abnormal levels of S100 beta protein can be detected and whether they could possibly serve as a marker for pre-clinical diagnosis.

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Markus Otto[1]
Michael Beekes[2]
Jens Wiltfang[3]
Erik Bahn[1]
Sigrid Poser[1]
Heino Diringer[2]
[1]*Neurologische Klinik und Poliklinik,*
Georg-August-Universität,
Robert-Koch Str. 40,
37075 Göttingen; Germany
[2]*Robert Koch-Institut (FG 123),*
Nordufer 20,
13353 Berlin, Germany
[3]*Psychiatrische Klinik und Poliklinik,*
Georg-August-Universität,
v. Siebold Str. 5,
37075 Göttingen, Germany

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