Reply

Reply by the authors

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No doubt, whether PrP106–126 exerts a toxic effect on neurons is an important question because of both the significance of prion research and the number of laboratories engaged in the study of this peptide. The working hypothesis of our investigation was that spontaneous chemical modifications of PrP106–126 might underlie its toxicity that had been reported by two different laboratories [1,2]. Indeed, we found Asn-108 of PrP106–126 to undergo spontaneous deamidation to aspartic acid and isoaspartic acid. The same residue is also deamidated and isomerized in full-length PrP [3]. However, we were not able to detect any neurotoxic activity neither in fresh nor aged preparations of PrP106–126. In the following, our response to Dr. Brown's criticism.

Dr Brown points out that we have not performed the controls he has made with a peptide corresponding to a scrambled PrP106–126 sequence or with neuronal cells from $PrP^{0/0}$ mice. Controls of this kind clearly are important if PrP106–126 has proven to be toxic. If, as in our case, no toxicity has been found with authentic PrP106–126 and with wild-type cells, these controls seem meaningless. We have performed a positive control, the Alzheimer amyloid β-peptide 25–35 proved to be toxic (Section 3.2. of paper). We fully agree with Dr. Brown that the finding of toxic components in the HPLC buffer is not relevant for the elucidation of the pathogenesis of prion diseases and for this reason we have not identified the responsible component.

Our handling of the peptides was designed to avoid microbial contamination and endotoxin production. In all experiments a stock solution was prepared from lyophilized peptide. The peptide was dissolved in water (10 mg/ml) and immediately passed through an 0.2-μm sterile filter. Measurement of the peptide concentration after filtration assured that the cell cultures received the indicated concentrations of the peptide. All further handling of peptide was executed under sterile conditions. The peptides readily formed fibrils (see Fig. 1). Fibril-containing preparations of the peptides also proved to be non-toxic (Section 2.1. and Fig. 1 of paper). We prepared the cell cultures according to the original published procedures. In these protocols, no co-cultures with microglial cells were used [1,2].

We received upon request peptide material from Dr. Kretzschmar. That sample from a new synthesis in their lab did not show a toxic effect in our hands, neither filtrated nor unfiltrated in cultures with added antibiotics. At the same time, Dr. Brown tested our peptide and obtained the results in Table 1 of his comment. These data correspond to his former report to us, i.e. that our peptide preparation was only 'slightly toxic' and that there was a difference in toxicity of the preparations from Cambridge and Zurich that needed an explanation. We are not convinced by Dr. Brown's present comments that in testing the various sets of conditions described in our paper we missed an important experimental variable. We would welcome the opportunity to supply other laboratories with samples of PrP106–126 or to test a convincing alternative protocol ourselves.

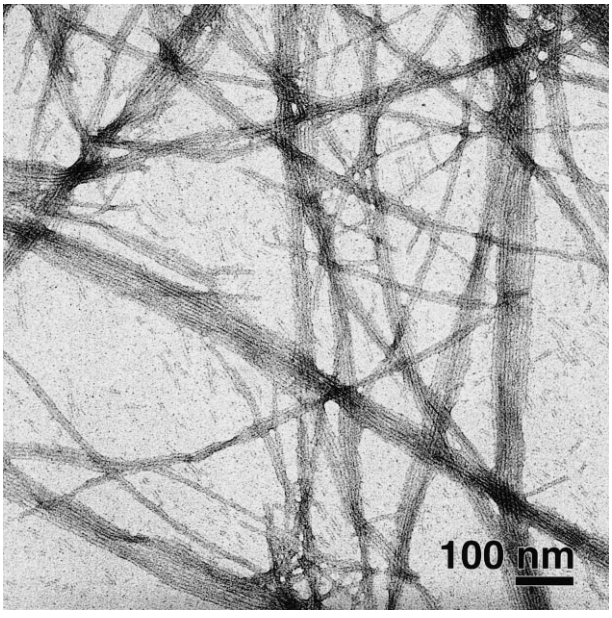

Fig. 1. Electron micrograph of PrP106–126 fibrils. The peptide (500 μM) was incubated in 0.1 M sodium phosphate pH 7.4 for 12 h at 37°C and then 10 times diluted with distilled water. Samples (5 μl) were applied to Formvar-carbon-coated grids, negatively stained with 1% uranyl acetate (w/v, in water), and examined with a Philips CM 100 transmission electron microscope at 80 kV. We thank Dr. Urs Ziegler for performing the electron microscopic analyses.

References

[1] Forloni, G., Angeretti, N., Chiesa, R., Monzani, E., Salmona, M., Bugiani, O. and Tagliavini, F. (1993) Nature 362, 543–546.
[2] Brown, D.R., Herms, J. and Kretzschmar, H.A. (1994) NeuroReport 5, 2057–2060.
[3] Sandmeier, E., Hunziker, P., Kunz, B., Sack, R. and Christen, P. (1999) Biochem. Biophys. Res. Commun. 261, 578–583.

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