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Detection of PrP^{res} in the Spleen of Hamsters Used as An in Vivo Model for Experimental TSE

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Abbreviations: i.p., intraperitoneal; MoAb, monoclonal antibody; TSE, Transmissible Spongiform Encephalopathies

INTRODUCTION

The pathogenesis of TSE has still not been completely clarified and discussed. In fact, in some animal species, other than neuroinvasion, accumulation of PrP^{re} in extraneural tissues has also been found, suggesting the possibility of concomitant lymphoinvasion. In particular, in experimentally infected mice, the lymphoreticular system and the spleen are infected for a long before neuroinvasion. Moreover, infection can be detected in the spleen 1 hour after intraperitoneal infection, suggesting that amplification of the pathogen in the spleen is necessary before neuroinvasion, at least for some scrapie strains (Beringue *et al*., 2000). In a study on prion diffusion in the hamster model, in animals intraperitoneally (i.p.) infected with the scrapie strain 263K, it was possible to identify a small amount of PrP^{res} in the spleen of the hamsters. Nevertheless, the concentration of PrP^{res} in the spleen showed no correlation with the level of PrP^{res} accumulation in any segment of the brain tissue. According to the authors, the spleen appeared to play a potential but non-essential role in pathogenesis after intraperitoneal infection in the hamster model (Baldauf *et al*., 1997). The aim of this work was to improve and validate an extraction method useful for quantification of the PrP^{res} in the spleen of prion-infected hamsters.

MATERIALS AND METHODS

20 outbreed Syrian Golden hamsters were used. 16 out of the 20 animals were infected i.p. with the scrapie strain 263 K by injecting 500 μl of brain homogenate containing 10^5 ID_{50}. Four hamsters were sacrificed at 70 days post-infection and the other animals at 1 week after the onset of clinical signs. The PrP^{res} extraction from the spleen of hamsters was performed, both with a method routinely used to confirm the diagnosis of BSE in brain tissues (Schaller *et al*., 1999) and with a method reported by Wadsworth *et al*. (2001); the second method

is based on the ability of sodium phosphotungstic acid (NaPTA), in the presence of $MgCl_2$ and at pH 7.4, to selectively precipitate PrP^{res} from extraneural tissues of vCJD patients. All the samples were digested with proteinase K at the final concentration of 50 μg/ml. The extracted prion protein was analysed in Western blotting, using both monoclonal antibody 3F4 (aa. 109–112) and SAF 70 (aa. 142–160).

RESULTS

The spleen of both the positive animal at the highest point of symptomatology and of the negative ones, extracted and analyzed with the method routinely used for diagnosis in brain tissues, failed to show any band related to the prion. On the contrary, in the spleens of infected animals extracted with NaPTA it was possible to identify a weak signal related to the di-glycosilated band (27–30 kDa), but only after 15 min of radiographic film exposure, suggesting the possibility of a false positive. The same method was applied to the whole spleens of 2 infected animals (1 at the terminal stage of the disease and 1 sacrificed 70 days post-infection) and allowed detection of the di-glycosilated band in both samples after 2 min of radiographic film exposure. Interestingly, in the infected animal at the terminal stage the spleen contains a greater quantity of PrP^{res} than in the animal sacrificed 70 days post-infection.

Since it has been demonstrated that the hamster spleen can be PrP^{res} negative despite the presence of PrP^{res} in the CNS (Baldauf *et al*., 1997), we also analysed the spleens of 8 animals at the terminal stage of disease at the same time. Furthermore, we used two different monoclonal antibodies (3F4 and SAF 70) to verify the specificity of the reaction.

The spleens were homogenized (10% w/v) in PBS containing 4% Sarcosyl and then centrifuged at 80 × *g* for 5 min. 500 μl samples of the resultant supernatant were incubated for 10 min at 37°C. The samples were then mixed with 50 U/ml of Benzonase and 1 mM $MgCl_2$ and incubated for 30 min at 37°C. NaPTA precipitation was performed by adding 40.65 μl of a stock solution containing 4% NaPTa and 170 mM $MgCl_2$ at pH 7.4. Samples were incubated at 37°C for 30 min with constant shaking before centrifugation at 15,800 × *g* for 30 min. The supernatant was discarded and the pellet resuspended in 200 μl of PBS containing 0.1% Sarcosyl and supplemented with 50 μl of 250 mM EDTA in order to eliminate white precipitates probably consisting of insoluble magnesium salts. Samples were then centrifuged again at 15,800 × *g* for 15 min. The pellets were resuspended with 50 μl of PBS containing 0.1% Sarcosyl and digested with proteinase K for 1 hour at 37°C with constant shaking; digestion was stopped by the addition of 1 μl 50 mM PMSF. Finally, after an additional centrifugation at 15,800 × *g* for 15 min, the pellets were resuspended to 20 μl final volume with Laemmli buffer, transferred to a 100°C heating block for 10 min and analyzed by Western blotting.

Two blots (4 samples/blot) were prepared: the first was blocked for 1 hour and incubated overnight at room temperature with the MoAb 3F4 diluted 1:25,000. The second blot was blocked overnight at 4°C and, after washing, was incubated for 1 hour at room temperature with the MoAb SAF 70 diluted 1:500. After a further incubation for 1 hour at room temperature with the secondary antibody, the reaction was developed with a chemiluminescent substrate (Immun-Star HRP chemiluminescent, Bio-Rad) for 2 min. As shown in Figure 1,

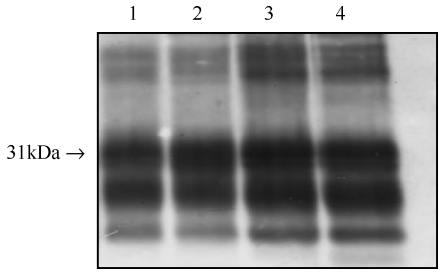

Figure 1. Western blotting detection of PrP^{res} in spleens of different i.p. infected hamsters with MoAb SAF 70. Lane 1–4 PrP^{res} detected starting from 50 mg of spleen tissue

the reaction with MoAb SAF 70 allowed detection of the PrP^{res} band with the expected pattern, quite identical to that obtained when using brain tissues.

DISCUSSION

In order to detect the presence of PrP^{res} in the spleen of infected hamsters, we used a newly developed extraction method, before Western blot analysis, allowing the precipitation of PrP^{res}. The NaPTA treatment applied before proteolysis causes a change in the PK cleavage site, resulting in the generation of larger PrP^{res} fragments, acting on the N-terminal region of PrP^{res}. By using this procedure, with some modifications, we succeeded in detecting the prion protein in the spleen and obtained the expected bands of prion protein, while in previous reports only very weak signals were obtained. Moreover, it was possible to confirm that the amount of detectable PrP^{res} in the hamster spleen is very low, suggesting that the spleen plays a non-essential role for development of the disease in the hamster experimental model.

REFERENCES

Beringue, V., Demoy, M., Lasmezas, C.I., Gouritin, B., Wewingarten, C., Deslys, J.P., Andreux, J.P., Couvreur, P. and Dormont, D., 2000. Role of spleen macrophages in the clearance of scrapie agent early in pathogenesis. *Journal of Pathology*, **190**(4), 495–502

Baldauf, E., Beekes, M. and Diringer, H., 1997. Evidence for an alternative direct rout of access for the scrapie agent to brain bypassing the spinal cord. *Journal of General Virology*, **78**, 1187–1197

Schaller, O., Fatzer, R., Stack, M., Clark, J., Cooley, W., Biffiger, K., Egli, S., Doherr, M., Vandevelde, M., Heim, D., Oesch, B. and Moser, M., 1999. Validation of a western immunoblotting procedure for bovine PrP(Sc) detection and its use as a rapid surveillance method for the diagnosis of bovine spongiform encephalopathy (BSE). *Acta Neuropathologica*, **98**(5), 437–443.

Wadsworth, J.D.F., Joiner, S., Hill, A.F., Campbell, T.A., Desbruslais, M., Luthert, P.J. and Collinge, J., 2001. Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. *The Lancet*, **358**, 171–180