(Q67L) and the transdominant negative (T22N) mutants for 48 h and analysed PrPSc levels. The differential effect of overexpression of these mutants on cholesterol metabolism in 22LN2a cells is analysed through Amplex cholesterol assay. Immunofluorescence experiments are conducted using an antibody against Rab7-GTP in prion infected and non-infected cell lines. Trafficking of LDL is analysed using by pulse-chase experiments with fluorescently labelled LDL and co-localization with endosomal markers.

Results: We demonstrate that in the prion infected cell lines, transient over-expression of the constitutively active mutant Rab7 reduces PrPSc propagation. Since prion infected cell cultures exhibit an increase in the cholesterol levels, we analysed how different functional mutants of Rab7 regulate cholesterol levels and observed that over-expression of the constitutively active Q67L mutant reduces the cholesterol level with respect to Rab7-WT mutant in 22LN2a cells. Overexpression of Rab7-WT did neither affect PrPSc levels, nor cholesterol content in those cells. This indicates that activation of Rab7 by GDP/GTP exchange might be impaired in 22LN2a cells. Preliminary results of a comparative analysis of Rab7-GTP in N2a and 22LN2a point at a reduction of Rab7-GTP upon prion infection. Since Rab7 is required for LDL transport, LDL trafficking is analysed to define a link between aberrant vesicle trafficking and enhanced cholesterol synthesis.

Conclusion: Through this study, we shed light on the underlying mechanisms and effects of impaired membrane association of Rab7 in prion infection. These effects include deterioration of cellular processes such as vesicle trafficking and cholesterol metabolism, hence favouring PrPSc propagation.

59. The role of glial cells in BSE pathology: a histomorphological and immunohistochemical investigation

Christine Fast[a], Kristina Santiago-Mateo[b], Rakhi Katoch[b], Martin H. Groschup[a], Ute Ziegler[a], Stefanie Czub[b]

[a]Friedrich-Loeffler Institut, Isle of Riems, Germany; [b]Canadian Food Inspection Agency, Lethbridge, Alberta, Canada

CONTACT Christine Fast Christine.Fast@fli.de

ABSTRACT

Background/Introduction: Bovine spongiform encephalopathy (BSE) is a fatal neurological disease of cattle associated with the accumulation of an altered form of the cellular prion protein, termed pathological prion protein (PrP^{BSE}) The pathomechanisms linking abnormal protein accumulation with the typical severe neurological alterations in BSE are less than clear. Here we investigated the role of glia cells in the pathology of BSE infected cattle, specifically if and to which degree the histopathological changes are glia mediated and therefore due to the host response and not to the BSE agent itself.

Material and Methods: We examined 29 experimentally BSE infected cattle at preclinical (no PrP^{BSE} in the brain stem), late preclinical (no clinical signs, first traces of PrP^{BSE} in the brain stem), and clinical (clinical signs and distinct accumulation of PrP^{BSE} in the brain stem) stages of the disease. The following defined regions of the CNS were examined: Obex, Red Nucleus, Cerebellum, Parietal Cerebrum, Septal Nucleus, and Hippocampus and both histopathological lesions and PrP^{BSE} accumulation were determined. Additionally, antibodies Iba-1, CX3CR1, and GFAP were used to visualize and identify glial involvement within the context of the disease. In addition, four unchallenged animals of the same age groups were included as negative controls.

Results: PrP^{BSE} is found only in neurons and microglia. Different analytic approaches showed that the most consistent changes in glial numbers and phenotypes were detected in the Obex, Red Nucleus, and Hippocampus. However, only in clinical affected cattle, we observed with all antibodies a mild diffuse increase in glial reactivity, indicating a gliosis, as compared to controls, preclinical, and late preclinical animals. Additionally, different glial reaction patterns were almost exclusively detected in clinical animals. This includes a hyperplastic and in parts irregular appearance of the glial network, an accumulation of clumsy cells with shortened processes and rounded cell bodies, a diffusely distributed coarse granular staining and occasionally perineuronal cluster of glial cells.

Conclusion: Apart from neurons, we identified microglia as the only other PrP^{BSE} accumulating cell population; computerized assessment of IBA1, CX3CR1, and GFAP antibodies are ongoing. The preliminary results presented here indicate that the activation of a glial response is a rather late event, most probably associated with the accumulation of PrP^{BSE}.

60. *Caribou* Prnp *polymorphism distribution in Canada and its impact on CWD pathogenesis*

Maria I. Arifin[a], Samia Hannaoui[a], Yuan-Hung Huang[a], Gordon Mitchell[b], Antanas Staskevicius[b], Lech Kaczmarczyk[c,d], Walker Jackson[c,d] and Sabine Gilch[a]

[a]Department of Ecosystem and Public Health, University of Calgary; [b]National and OIE Reference Laboratory for Scrapie and CWD, Ottawa Laboratory Fallowfield, Canadian Food Inspection Agency; [c]Wallenberg Center for Molecular Medicine, Department of Clinical