Evidence for a Cysteine Proteinase Involved in BSE Infection and/or Progression

Viral nucleic acid sequences and proteinases are potentially involved in bovine spongiform encephalopathy disease infection and/or progression.

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Introduction

According to the "protein-only" hypothesis, the prion protein seemed to be – for more than a decade – the infectious agent of transmissible spongiform encephalopathies (TSEs) in different species [1]. TSEs are neurodegenerative diseases reported *e.g.*, for humans (Creutzfeld-Jakob disease, CJD), cattle (bovine spongiform encephalopathy, BSE), and sheep (scrapie). In contrast to the "protein-only" hypothesis, some researchers published data showing that retrovirus-like RNA transcripts [2], a viral structure [3], and viral particles [4] might be involved in disease infection/progression of CJD. In addition, there is no final proof for the "protein-only" hypothesis and the infectivity of the prion protein. Recent research has questioned the "protein-only" hypothesis again and proposes intracellular 25-nm virus-like particles as the causal TSE virions that induce late-stage prion protein brain pathology [5].

The goal of this research study was to test for virus-like particles that are involved in infection/progression of BSE. Whole blood samples of experimentally and naturally BSE-infected and healthy cattle were analyzed with PCR primers specific for common nucleic acid sequences of different groups of viruses. Nucleic acid markers for BSE were quantitatively analyzed by real-time PCR using the LightCycler® System.

Materials and Methods

Samples

DNA from whole blood samples of three different groups of cattle were prepared: (1) blood taken at lifetime from ten cows tested postmortem BSE positive by western blotting, (2) seven experimentally infected animals (oral application of BSE-contaminated brain tissue homogenate), and (3) sixteen BSE-negative controls.

Treatment of whole blood samples

Blood cells were lysed by adding two volumes of RNA/DNA Stabilization Reagent for Blood/Bone Marrow (50 ml whole blood + 100 ml stabilization reagents). Immediately after vigorous shaking the samples were frozen in liquid nitrogen and subsequently stored at -80°C.

Preparation of nucleic acids

During thawing of lysed bovine whole blood samples at room temperature, 150 mg dithiothreitol and 100 ml lysis/binding buffer of MagNA Pure LC Total Nucleic Acid Isolation Kit were added. Depending on the type of nucleic acid of interest, mRNA or total nucleic acid was purified using the MagNA Pure LC System and the respective purification reagents and protocols.

Amplification of specific sequences by real-time PCR

Real-time PCR experiments were run on the LightCycler® Carousel-Based System. Reverse transcriptase (RT) PCR was performed using LightCycler® RNA Master SYBR Green I. For amplification of DNA the LightCycler® FastStart DNA Master^{PLUS} SYBR Green I was used. Primers for different groups of viruses were designed to test for a broad range of viruses potentially involved in BSE disease infection/progression (*i.e.*, moloney murine leukemia virus, bovine herpesvirus, bovine parvovirus, visna lentivirus).

Analysis of results

Quantitative analysis of specific PCR products was based on real-time PCR/RT-PCR data generated by the LightCycler® Carousel-Based System. Amplicon sizes of PCR products were analyzed by gel electrophoresis. Bioinformatic analysis of sequences was performed at the Roche Bioinformatics Basel Homepage using "NUC wgs_cow — whole genome shotgun, Bos Taurus, Bovine Sequencing Consortium" and databases of the National Center for Biotechnology Information (NCBI).

Results and Applications

Analysis of PCR fragments

Primers specific for the long terminal repeat (LTR) region quantitatively discriminated BSE-infected and healthy

cattle. The respective fragment size of the PCR products was analyzed by gel electrophoresis. Figure 1 shows a fragment of 255 bp in samples of postmortem BSE-positive cattle (samples "FC"), experimentally infected (samples "EI"), and in samples of animals showing clinical signs of BSE (samples "VF"). Control samples were negative except for those two samples of the two healthy cattle with only weak signals in the range of about 250 bp ("SH9", "SH17").

The 255-bp PCR fragment identified by LTR-specific primers was sequenced. Based on this sequence, specific primers for the 255-bp PCR fragment were designed and further quantitative RT-PCR experiments were performed.

Table 2 documents the results of a typical experiment with normalized RNA amounts of all samples. Earlier crossing point (CP) values were found for BSE-infected compared with healthy animals (difference of mean CP values 1.7).

To identify the 255-bp PCR fragment, the sequence was blasted against cow DNA/RNA sequence databases that were published at the time of the study. No extended matching sequences were found. This result revealed the sequence as new in cows. By searching for homologies in other sequence databases, a motif coding for 18 amino acids in the region of the enzymatic center of a cysteine proteinase was identified. Cysteine proteinases have already been described as playing a critical role in processes of another neurodegenerative disease – Alzheimer's Disease [6, 7].

Conclusions

The results of these experiments show that virus-like nucleic acids and/or proteinases might be involved in infection and/or progression of BSE. Further research in this field might help to understand BSE infection/progression and might further question the "protein-only" hypothesis of TSE diseases. ■

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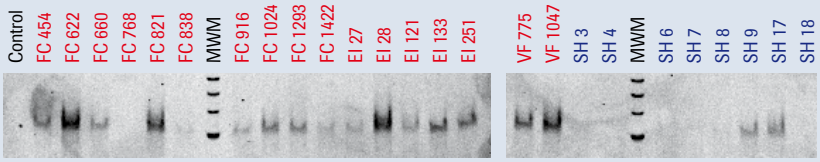

Figure 1: RT-PCR analysis identified a 255-bp fragment to be specific for BSE-infected cattle and animals with clinical signs (red). Healthy animals are labeled in blue. (Control: no template control; MWM: molecular weight marker).

Table 2: Specific primers for the 255-bp fragment discriminate between BSE-infected and healthy cattle. **(a)** CP values for ten infected cattle (postmortem confirmed BSE positive). **(b)** CP values for eight healthy cattle (postmortem BSE negative).

a) Sample No.	Status	CP values
1	BSE-infected	32.49
2	BSE-infected	32.93
3	BSE-infected	33.94
4	BSE-infected	33.53
5	BSE-infected	33.94
6	BSE-infected	33.76
7	BSE-infected	31.91
8	BSE-infected	33.04
9	BSE-infected	33.12
10	BSE-infected	33.78
BSE-infected cattle, mean value		**33.2**
b) Sample No.	**Status**	**CP values**
1	Healthy	35.46
2	Healthy	35.52
3	Healthy	34.07
4	Healthy	34.06
5	Healthy	36.50
6	Healthy	34.04
7	Healthy	35.50
8	Healthy	34.19
Healthy cattle, mean value		**34.9**

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