別紙様式第2-1

医薬品 研究報告 調査報告書

<table>
<tr><td>識別番号・報告回数</td><td></td><td></td><td>報告日</td><td>第一報入手日
2005. 11. 24</td><td colspan="2">新医薬品等の区分
該当なし</td><td>機構処理欄</td></tr>
<tr><td>一般的名称</td><td colspan="2">人血清アルブミン</td><td rowspan="2">研究報告の公表状況</td><td rowspan="2">Ligios C, Sigurdson CJ, Santucciu C, Carcassola G, Manco G, Basagni M, Maestrale C, Cancedda MG, Madau L, Aguzzi A. Nat Med. 2005 Nov;11(11):1137-8.</td><td colspan="2">公表国</td><td rowspan="2"></td></tr>
<tr><td>販売名(企業名)</td><td colspan="2">赤十字アルブミン20(日本赤十字社)
赤十字アルブミン25(日本赤十字社)</td><td colspan="2">イタリア</td></tr>
<tr><td>研究報告の概要</td><td colspan="6">○スクレイピーおよび乳腺炎に罹患した羊の乳腺におけるPrP^{Sc}
イタリアのサッサリ県で818頭の羊を調査した。7頭は脳、リンパ節、扁桃腺においてPrP^{Sc}が検出され、臨床的に明らかなスクレイピーの症状を呈していた。4頭が乳腺炎とスクレイピーを併発していた。この4頭全ての乳腺においてPrP^{Sc}が検出されたが、乳腺炎を併発していないスクレイピー発症前の羊やスクレイピーを発症した同じ群(n=14)又は他の群(n=1)由来の羊、乳腺炎に罹患しているがスクレイピーへの感染は認められない羊(n=2)においてはPrP^{Sc}は検出されなかった。乳腺の炎症病変部の解析では、PrP^{Sc}のリンパ濾胞部位への集積が認められた。PrP^{Sc}は、乳腺炎による病変部位中の主にCD68+マクロファージおよびFDCsと共局在化していた。
慢性的な炎症とスクレイピーの併発により、PrP^{Sc}が想定外の組織まで拡大して蓄積する可能性が示された。乳房中のPrP^{Sc}濃度の中央値は、脾臓の0.1%、脳の0.05%と算出されたが、乳房のリンパ濾胞は確率的な分布を示しているため、局部的なPrP^{Sc}量には顕著なばらつきが認められた。
本研究ではMaedi-Visnaウィルス(MVV)の血清抗体陽性反応とリンパ濾胞乳腺炎の相関が示された。ヨーロッパの小型反芻動物のほとんどはMVVおよび関連レンチウィルスに感染している。ごく一般的なウィルス感染が原因となるプリオン病感染拡大の可能性が示唆された。MVVは、乳房上皮細胞やマクロファージ中に存在し、羊乳を介して子羊に伝播することが実験的に証明されている。PrPの乳房リンパ濾胞部位のCD68+細胞への蓄積は、乳腺炎の羊の羊乳中への大量のマクロファージの混入も併せ、プリオン感染と分泌器官の炎症の併発が分泌物のプリオン汚染を誘導し、群中におけるプリオンの水平感染の共同因子となり得るのか、という疑問を提起することとなった。</td><td>使用上の注意記載状況・その他参考事項等
赤十字アルブミン20
赤十字アルブミン25

血液を原料とすることに由来する感染症伝播等</td></tr>
<tr><td colspan="3">報告企業の意見</td><td colspan="4">今後の対応</td><td></td></tr>
<tr><td colspan="3">スクレイピーおよび乳腺炎に罹患した羊の乳腺でPrP^{Sc}が検出されたとの報告である。</td><td colspan="4">これまでの疫学研究等では、ヒトにおいて、血漿分画製剤を介してスクレーピーを含む伝達性海綿状脳症(TSE)が伝播するという証拠はない。また異常プリオンがアルブミン製剤の製造工程で効果的に除去されるとの報告もあるが、輸血によりvCJDに感染する可能性が示唆されたことから、今後も情報の収集に努める。</td><td></td></tr>
</table>

MedDRA/J Ver.8.1J

Table 1 Summary of the fluorescence correlation spectroscopy measurements using 10 μM RITA

Protein	Diffusion time ± s.e.m.[a]	Change in diffusion time, percent
No protein	0.063 ± 0.011	—
GST-p53 dN(1–63)	0.356 ± 0.070	465
GST-p53 N(1–100)	0.259 ± 0.020	311
GST-p53(1–393)	0.287 ± 0.043	355
His-p53(1–393)	0.198 ± 0.007	214
His-p53(1–312)	0.111 ± 0.014	74
GST	0.076 ± 0.003	20
GST-EBNA2	0.073 ± 0.017	16

[a]All experiments were performed at least three times.

subject for future research.

Accession codes. BIND identifiers (http://bind.ca): 335735.

Vera Grinkevich[1], Natalia Issaeva[1], Shakil Hossain[2], Aladdin Pramanik[2] & Galina Selivanova[1]

*[1]Microbiology and Tumor Biology Center and [2]Department of Medical Biochemistry and Biophysics, Karolinska Institutet, SE-17177 Stockholm, Sweden.
e-mail: galina.selivanova@mtc.ki.se*

1. Issaeva, N. *et al. Nat. Med.* 10, 1321–1328 (2004).

© 2005 Nature Publishing Group http://www.nature.com/naturemedicine

PrP^Sc in mammary glands of sheep affected by scrapie and mastitis

To the editor:
Besides colonizing the central nervous system, the infectious agent of transmissible spongiform encephalopathies, termed prion, is predominantly associated with follicular dendritic cells (FDCs) of lymphoid tissues[1,2]. Accordingly, PrP^Sc, a protease-resistant isoform of the host protein PrP^C representing the main prion constituent, is often detectable in spleen, tonsils, Peyer patches and lymph nodes of infected hosts.

Chronic inflammatory states are accompanied by local extravasation of B cells and other inflammatory cells, which may induce lymphotoxin-dependent maturation of ectopic FDCs. Consequently, scrapie infection of mice suffering from nephritis, hepatitis or pancreatitis induces unexpected prion deposits at the sites of inflammation[3]. This has raised concerns that analogous phenomena might occur in farm animals.

We have investigated this question in a flock of 818 Sarda sheep held in the Sassari region of Italy for production of wool and human foods. The European Surveillance Plan for Transmissible Spongiform Encephalopathies mandates the removal of all sheep of scrapie-susceptible genotypes in scrapie-infected flocks.Of the 818 sheep, 261 had *Prnp* alleles[4] that conferred susceptibility to prion disease. Of the latter, seven had clinically overt scrapie with PrP^Sc in brain, lymph nodes and tonsil. All scrapie-sick sheep and 100 randomly chosen healthy sheep were killed, and mammary glands were analyzed histologically. Of these, 10 sheep had lymphocytic mastitis, and four had coincident mastitis and scrapie. Using western blots, immunohistochemistry and histoblots, we detected PrP^Sc in mammary glands of all four clinically scrapie-sick sheep with mastitis (Fig. 1a,b), but not in noninflamed mammary glands from presymptomatic or scrapie-sick sheep from the same (n = 14) or a different flock (n = 1), nor in inflamed mammary glands of scrapie-uninfected sheep (n = 2). Within the inflammatory mammary lesions, PrP^Sc was found to be associated with lymphoid follicles by immunofluorescent labeling and by paraffin-embedded tissue (PET) blotting (Fig. 1c). PrP^Sc colocalized predominantly with CD68$^+$ macrophages and FDCs within inflamed mammary glands (Fig. 2a).

We then surveyed a second Sarda flock (272 sheep) located 30 km away from the flock described above. One sheep was found to be

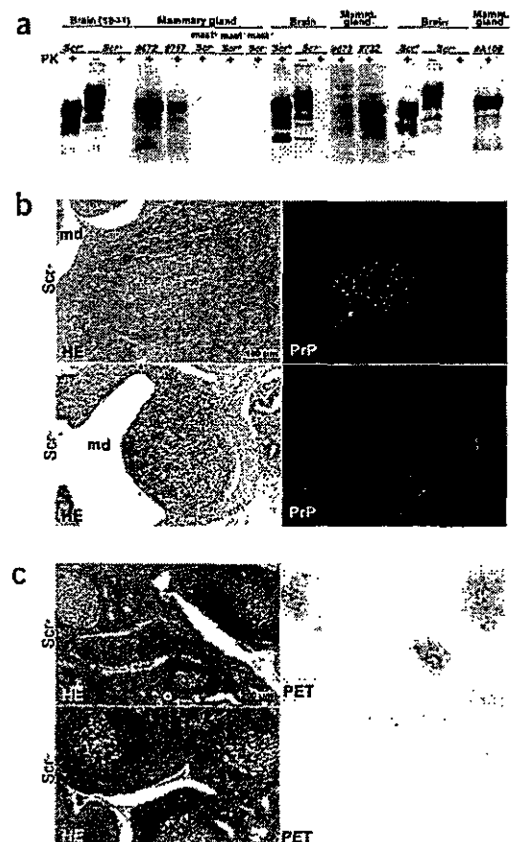

Figure 1 Prion protein in inflamed mammary glands. (a) Western blots with a PrP-specific antibody. Lanes 1–3, 9–11, 14–16 from left: native and proteinase K (PK)-digested brain homogenates (diluted 1/1,400) from a scrapie-infected (Scr$^+$) and a scrapie-free sheep (Scr$^-$). Lanes 6–8: mammary glands from a scrapie-free sheep with follicular mastitis (Scr$^-$, mast$^+$), a scrapie-positive sheep from a flock with neither MVV seropositivity nor mastitis (Scr$^+$, mast$^-$), and a sheep with neither mastitis nor scrapie (Scr$^-$, mast$^-$). Each one of five scrapie-infected sheep with mastitis had mammary PrP^Sc (lanes 4, 5, 12, 13, and 17). Non-scrapie-infected brain and mammary gland extracts showed no PrP^Sc upon PK digestion (lanes 3, 6–8, 11 and 16). (b) Mammary gland micrographs from MVV-seropositive sheep with mastitis and coincident scrapie (Scr$^+$), or with mastitis but no scrapie (Scr$^-$). Lymphoid follicles are adjacent to milk ducts (md). Immunofluorescence stains show abundant PrP deposits within mammary lymphoid follicles (arrow) from scrapie-positive but not from scrapie-free sheep. Scale bars, 100 μm. (c) PK-treated paraffin-embedded tissue blots of mammary gland sections show punctate PrP^Sc deposits colocalizing with lymphoid follicles in scrapie-infected (Scr$^+$), but not in scrapie-free (Scr$^-$) sheep with mastitis. Scale bars, 200 μm.

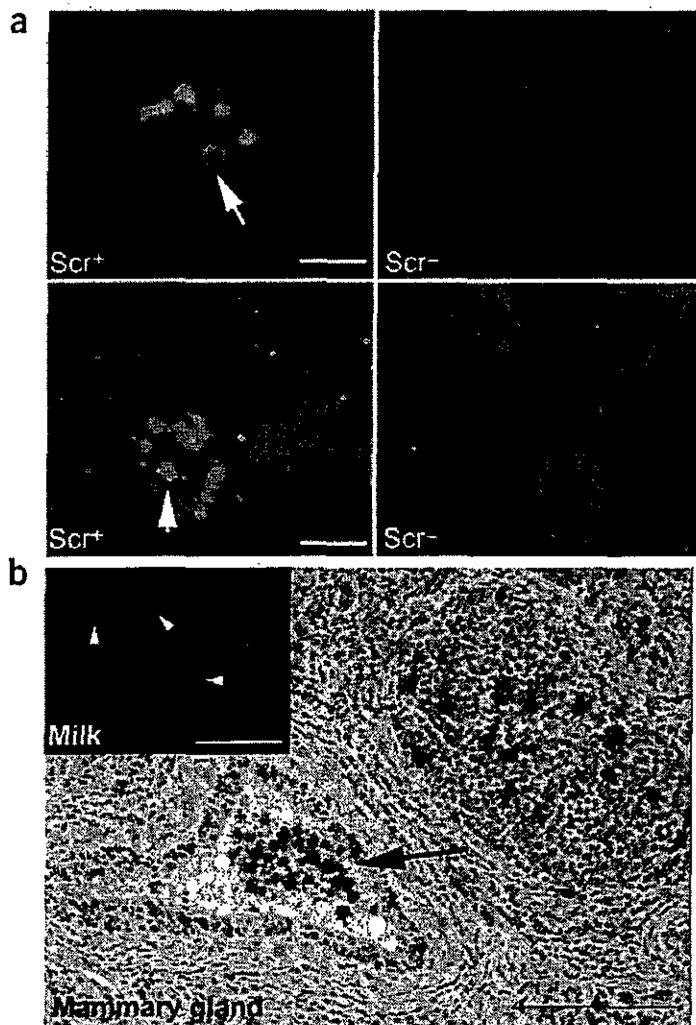

Figure 2 Mammary PrP^{Sc} localizes to macrophages and FDCs. (a) Mammary gland from a sheep with coincident mastitis, MVV seropositivity and scrapie (sheep #732). Confocal laser scanning micrographs of lymphoid follicles immunostained for PrP (green), nuclear DNA (blue) and macrophages (red, top panels) or FDC (red, bottom panels). PrP^{Sc} associates with CD68+ macrophages and FDCs in scrapie-positive (Scr+, arrows) but not in scrapie-free sheep (Scr−). Scale bars, 6.3 μm (top) and 7.5 μm (bottom). (b) CD68+ macrophages (arrow) and degenerating leukocytes within milk ducts and in adjacent lymphoid follicles of an inflamed mammary gland, as well as in milk sediment (inset, arrowheads). Scale bar, 100 μm (mammary gland) or 20 μm (milk cells).

scrapie-sick and was killed: necropsy showed lymphofollicular mastitis and PrP^{Sc} in the brain and tonsil. Again, PrP^{Sc} was present in the mammary gland (Fig. 1a). These results indicate that coincidence of natural chronic inflammatory conditions and natural scrapie can expand the deposition of PrP^{Sc} to unexpected tissues of sheep.

By plotting western blot signals against serially diluted scrapie-infected brain and spleen, we determined that the median mammary PrP^{Sc} concentration was 0.1% of that of spleen and 0.05% of brain. But because mammary lymphoid follicles were stochastically distributed, local PrP^{Sc} loads varied markedly. Hence these figures may underestimate PrP^{Sc} in sites of abundant follicles, and overestimate it in sites with few or no follicles.

Common causes of lymphofollicular mastitis in sheep include Maedi-Visna virus (MVV) and mycoplasma[5]. We could not culture mycoplasma from mastitic glands, whereas we found that four of the five sheep with scrapie and mastitis were seropositive for MVV and that the three scrapie-sick sheep without mastitis were seronegative for MVV. In the clinically healthy group, 7 of 10 sheep with mastitis, but only 32 of 90 sheep without mastitis, were seropositive for MVV. Hence, MVV seropositivity correlated with lymphoid follicular mastitis (Fisher exact test, $P = 0.01$) as reported previously[6,7].

MVV and related small-ruminant lentiviruses are endemic in most, if not all, European populations of small ruminants[6]. The above data suggest that common viral infections of small ruminants may enhance the spread of prions. MVV is found within mammary epithelial cells and macrophages[8], and has been experimentally passed to lambs through milk[9]. Milk is believed to represent a major route of transmission for the natural spread of MVV[5]. The PrP deposits in CD68+ cells of mammary lymphoid follicles, in concert with the copious shedding of macrophages into milk of mastitic sheep (Fig. 2b)[9,10], raises the question whether coexistence of prion infection and inflammation in secretory organs may lead to prion contamination of secretes, and may represent a cofactor for horizontal prion spread within flocks.

ACKNOWLEDGMENTS
We thank J. DeMartini for discussions and B. Seifert for statistical analysis. This study was supported by grants of the European Union (Apopis), the Swiss National Foundation, the National Competence Center for Research on Neural Plasticity and Repair (to A.A.), Istituto Zooprofilattico Sperimentale della Sardegna 001/02 (to C.L.), US National Institutes of Health K08-AI01802 and the Foundation for Research at the Medical Faculty, University of Zürich (to C.J.S.) and the United States National Prion Research Program (to C.J.S. and A.A.).

Ciriaco Ligios[1,6], Christina J Sigurdson[2,6], Cinzia Santucciu[1,6], Gabriella Carcassola[3], Giuseppe Manco[2], Massimo Basagni[4], Caterina Maestrale[1], Maria Giovanna Cancedda[5], Laura Madau[1] & Adriano Aguzzi[2]

[1]Istituto Zooprofilattico Sperimentale della Sardegna, I-07100 Sassari, Italy. [2]UniversitätsSpital Zürich, Institute of Neuropathology, Department of Pathology, CH-8091 Zürich, Switzerland. [3]Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria, Sez. di Microbiologia e Immunologia, Università degli Studi di Milano, I-20133 Milano, Italy. [4]Prion DGN Srl, I-20017 Rho, Italy. [5]Istituto Zootecnico Caseario della Sardegna, I-07040 Olmedo, Italy. [6]These authors contributed equally to this work.
e-mail: adriano@pathol.unizh.ch

1. Mabbott, N.A., Mackay, F., Minns, F. & Bruce, M.E. *Nat. Med.* **6**, 719–720 (2000).
2. Montrasio, F. *et al. Science* **288**, 1257–1259 (2000).
3. Heikenwalder, M. *et al. Science* **307**, 1107–1110 (2005).
4. Goldmann, W. *et al. Proc. Natl Acad. Sci. USA* **87**, 2476–2480 (1990).
5. Pepin, M., Vitu, C., Russo, P., Mornex, J.F. & Peterhans, E. *Vet. Res.* **29**, 341–367 (1998).
6. Peterhans, E. *et al. Vet. Res.* **35**, 257–274 (2004).
7. de Andres, D. *et al. Vet. Microbiol.* **107**, 49–62 (2005).
8. Carrozza, M.L., Mazzei, M., Bandecchi, P., Arispici, M. & Tolari, F. *J. Virol. Methods* **107**, 121–127 (2003).
9. Preziuso, S. *et al. Vet. Microbiol.* **104**, 157–164 (2004).
10. Lerondelle, C. & Ouzrout, R. *Dev. Biol. Stand.* **72**, 223–227 (1990).

© 2005 Nature Publishing Group http://www.nature.com/naturemedicine

別紙様式第 2-1

別紙 3-4

医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2005 年 10 月 25 日	新医薬品等の区分 該当なし		厚生労働省処理欄
一般的名称			研究報告の公表状況	Ultra-sensitive detection of prion protein fibrils by flow cytometry in blood from cattle affected with bovine spongiform encephalopathy Lothar Trieschmann, Alexander Navarrete Santos, Katja Kaschig, Sandra Torkler, Elke Maas, Hermann Schätzl and Gerald Böhm* BMC Biotechnology 2005, 5:26	公表国 ドイツ		
販売名（企業名）							

研究報告の概要	使用上の注意記載状況・その他参考事項等
現在、BSE、CJD、CWD、そしてスクレイピーなどの伝染性海綿状脳症 (TSE) の確定診断は、死後の病理解剖で病原性プリオン (PrPsc) が検出されることによってのみ下される。一方、臨床症状を発現する前でも、血液中に PrPsc が存在する可能性が示唆されており、輸血などによる伝播が問題となっている。しかし PrPsc の血中濃度は非常に低く、現在の分析法では検出することが困難である。著者らは、「核」を加えた場合と加えなかった場合のプリオン蛋白単量体の重合化動態の違いを利用して、高感度のプリオン蛋白凝集体の検出法を開発した。凝集の検出はフローサイトメトリーにより行った。BSE を発症した 6 頭のウシおよび 4 頭の正常ウシの血清を使用して実験を行った。予め作成した、蛍光標識したプリオン単量体を血清に添加すると、添加しなかった場合と比較して、この単量体が「核」となり新たな凝集塊の形成が促進されることが確認された。この実験系では、発症ウシの血清 6 検体全てにおいて、10^{-8}nM (0.24fg/mL) の低濃度でも合成プリオンの凝集塊が検出された。以上の結果から、本検出法は、少量の BSE 陽性の血清と正常血清とを識別する方法として有望であり、プリオン病の発症前診断の手がかりとなるかもしれない。	BYL-2005-0199

報告企業の意見	今後の対応
弊社の血漿分画製剤の製造工程におけるプリオン除去能は 4 log を上回ることが確認されている。本論文の実験結果が実用化されれば，プリオンの理論的伝播リスクがさらに低減することが期待される。	現時点で弊社において新たな安全対策上の措置を講じる必要はないと考える。引き続き本方法の実用化および PrP^{sc} の検出・除去技術に関する関連情報の収集に努める。

BYL-2005-0199

BMC Biotechnology

BioMed Central

Research article

Ultra-sensitive detection of prion protein fibrils by flow cytometry in blood from cattle affected with bovine spongiform encephalopathy

Lothar Trieschmann[1], Alexander Navarrete Santos[1], Katja Kaschig[1], Sandra Torkler[1], Elke Maas[2], Hermann Schätzl[2] and Gerald Böhm*[1]

Address: [1]ACGT ProGenomics AG, Weinbergweg 22, D-06120 Halle (Saale), Germany and [2]Institute of Virology, Technical University of Munich, Biedersteinerstrasse 29, D-80802 Munich, Germany

Email: Lothar Trieschmann - lothar.trieschmann@acgt-progenomics.de; Alexander Navarrete Santos - alexander.navarrete.santos@acgt-progenomics.de; Katja Kaschig - katja.kaschig@acgt-progenomics.de; Sandra Torkler - sandra.torkler@acgt-progenomics.de; Elke Maas - maas@lrz.tu-muenchen.de; Hermann Schätzl - schaetzl@lrz.tum.de; Gerald Böhm* - gerald.boehm@acgt-progenomics.de

* Corresponding author

Published: 04 October 2005

BMC Biotechnology 2005, 5:26 doi:10.1186/1472-6750-5-26

Received: 03 May 2005
Accepted: 04 October 2005

This article is available from: http://www.biomedcentral.com/1472-6750/5/26

© 2005 Trieschmann et al; licensee BioMed Central Ltd.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Background: The definite diagnosis of prion diseases such as Creutzfeldt-Jakob disease (CJD) in humans or bovine spongiform encephalopathy (BSE) in cattle currently relies on the *post mortem* detection of the pathological form of the prion protein (PrP^{Sc}) in brain tissue. Infectivity studies indicate that PrP^{Sc} may also be present in body fluids, even at presymptomatic stages of the disease, albeit at concentrations well below the detection limits of currently available analytical methods.

Results: We developed a highly sensitive method for detecting prion protein aggregates that takes advantage of kinetic differences between seeded and unseeded polymerization of prion protein monomers. Detection of the aggregates was carried out by flow cytometry. In the presence of prion seeds, the association of labelled recombinant PrP monomers in plasma and serum proceeds much more efficiently than in the absence of seeds. In a diagnostic model system, synthetic PrP aggregates were detected down to a concentration of approximately 10^{-8} nM [0.24 fg/ml]. A specific signal was detected in six out of six available serum samples from BSE-positive cattle.

Conclusion: We have developed a method based on seed-dependent PrP fibril formation that shows promising results in differentiating a small number of BSE-positive serum samples from healthy controls. This method may provide the basis for an *ante mortem* diagnostic test for prion diseases.

Background

A group of fatal transmissible neurodegenerative diseases, including Creutzfeld-Jakob disease (CJD), bovine spongiform encephalopathy (BSE), chronic wasting disease (CWD) and scrapie, is caused by an unusual infectious agent that has been termed prion [1]. Prions consist of an aberrant isoform (PrP^{Sc}) of the normal cellular prion protein (PrP^{C}). PrP^{C} is a cell surface glycoprotein expressed in neurons [2] and other cell types [3,4]. The precise physiological function of the cellular prion protein is not known yet. PrP^{Sc} differs from PrP^{C} in its higher content of β-sheet structure [5,6], its partial resistance to protease digestion

[7], and its tendency to form large aggregates [8]. PrP^{Sc} propagates by converting the cellular prion protein to the PrP^{Sc} conformation [9]. PrP^{Sc} aggregates accumulate predominantly in the central nervous system (CNS), and definitive diagnosis of prion diseases currently relies on the *post mortem* detection of PrP^{Sc} in CNS tissue by immunohistochemistry, Western blotting, or ELISA [10]. Transmission studies indicate that prions may also be present in blood, potentially allowing for *ante mortem* diagnosis, but the sensitivity of the currently available analytical methods is insufficient for the detection of the extremely low prion titers that can be expected in body fluids [11].

Here, we report the development of a method based on kinetic differences between seeded and unseeded aggregation of prion protein that allows the detection of PrP aggregates in blood down to attomolar concentrations by flow cytometry.

Results and discussion

Detection of synthetic prion protein aggregates in serum or plasma

Kinetic differences between seeded and spontaneous polymerization of peptide monomers can be used for the detection of amyloid β-protein aggregates in the cerebrospinal fluid of Alzheimer's disease patients [15]. Here, we extend the principle of seeded polymerization to the detection of prion protein aggregates.

While trying to establish conditions for the labeling of synthetic prion protein aggregates with a fluorescently labeled prion protein probe, we observed that the formation of prion protein aggregates proceeds much less efficiently in serum or plasma (not shown) than in PBS (Fig. 1). This inhibition is probably caused by interactions of the prion protein probe with serum proteins.

Next, we found that the addition of preformed prion protein aggregates to plasma can partially overcome this inhibition (Fig. 2). The preformed aggregates presumably function as seeds that facilitate the formation of new aggregates in the inhibitory environment of plasma. The seeds stimulated the formation of prion protein aggregates at all concentrations tested, from 5 nM [120 ng/ml] to 10^{-8} nM [0.24 fg/ml] (Fig 2C). The average ratio of event counts in seeded samples to those in samples without seeds was 6.4. The number of events, however, was not proportional to the seed concentration, but remained relatively constant over the whole concentration range. Thus, the seed-dependent formation of prion protein aggregates can be used to detect extremely low amounts (down to the attomolar range) of spiked prion protein aggregates in blood.

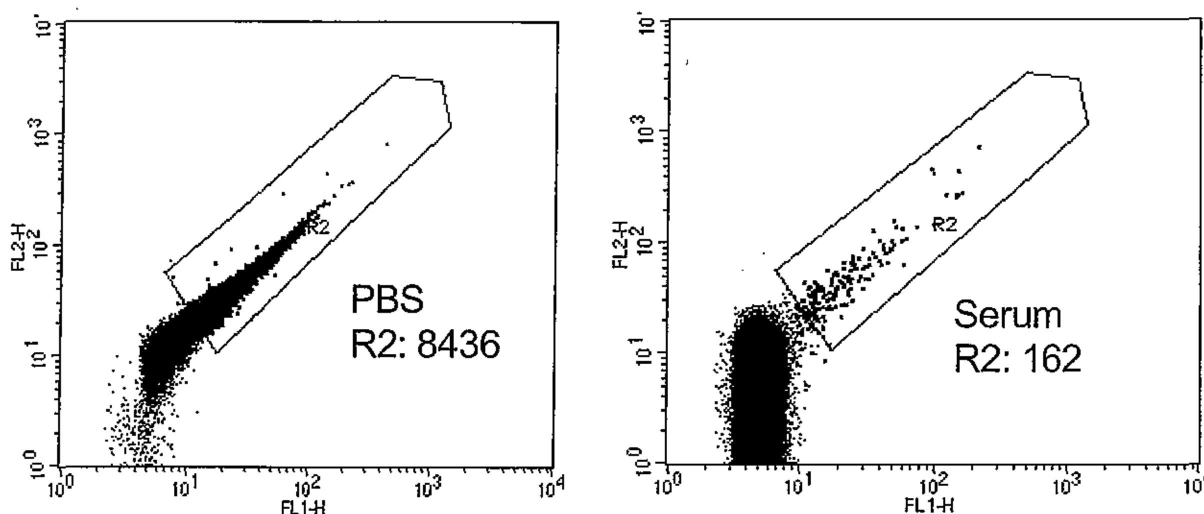

Figure 1
Inhibition of PrP aggregation in serum. FITC-labeled recombinant bovine prion protein (concentration 10 nM) was incubated at 37°C for 20 h with continuous shaking, either in 150 µl PBS (left panel) or in the same volume of serum (right panel), followed by flow cytometry. The measurements are depicted in a Fluorescence 1 (FL1-H) vs. Fluorescence 2 (FL2-H) dot-plot. The number of counts in the area containing specific signals (R2) is given in the figures. Aggregate formation in serum is strongly inhibited.

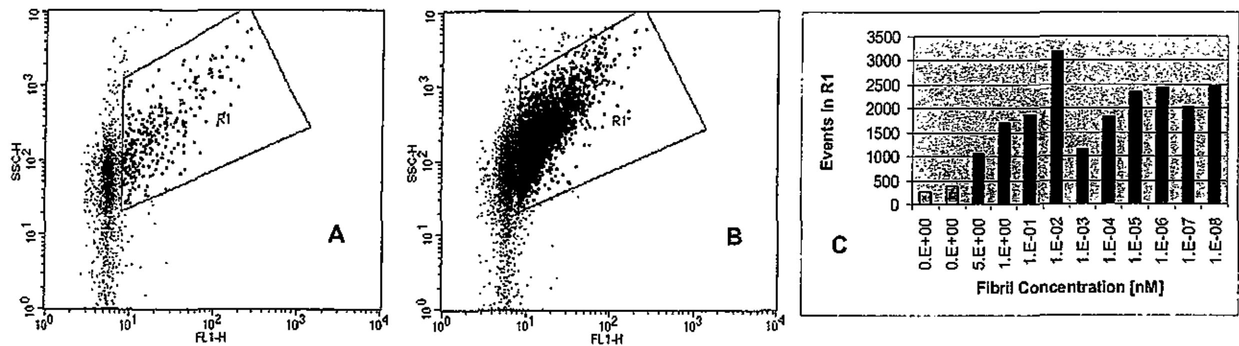

Figure 2
Seed-dependent PrP aggregate formation in plasma. FITC-labeled recombinant prion protein (5 nM) was incubated in plasma as described in the methods section for 20 h either in the absence (panel A) or presence (panel B) of 10^{-8} nM PrP aggregates. Panel C: quantification of measurements shown in A and B, and of measurements (not shown) with different seed concentrations. The measurements are depicted in a Fluorescence 1 (FL1-H) vs. Side-Scatter (SSC) dot-plot. Aggregate formation (signal in region R1) was strongly enhanced by all seed concentrations tested, from 5 nM to 10^{-8} nM.

Analysis of serum from clinical-stage, BSE-positive cattle

Studies demonstrating the transmission of prion diseases by blood transfusion suggest that prions are present in the blood of afflicted animals and people, even at pre-symptomatic stages of the disease [16-18]. We used the method of seed-dependent fibril formation to analyze serum from six confirmed cases of clinical-stage, BSE-positive cattle and four controls. Based on the spiking experiments described above, our hypothesis was that any PrP^{Sc} aggregates present in serum may act as seeds for the formation of easily detectable amounts of labeled PrP aggregates, whereas in the absence of seeds the formation of PrP aggregates would be inhibited. The serum samples from BSE-positive cattle and controls from healthy cattle were incubated with 10 nM of a FITC-labeled bovine PrP probe at 37°C for 20 h with continuous shaking, followed by analysis in a flow cytometer. All six BSE-samples could be clearly distinguished by a population of events that was absent in the controls (Fig. 3A–J, green dots in region R3; quantification in fig. 3K).

Conclusion

We have developed a method based on seed-dependent PrP fibril formation that shows promising results in differentiating a small number of BSE-positive serum samples from healthy controls. More samples need to be tested in order to validate its potential as an *ante mortem* diagnostic test for BSE and other prion diseases.

Methods

Biological fluids

Serum samples from six confirmed cases of BSE in cattle and four control animals were obtained from BFAV, Insel Riems, Germany. Control plasma was obtained from a blood bank.

Labeling of prion protein

Recombinant full-length bovine PrP was produced as described previously [12,13]. The purified protein was labeled with a FITC-labeling kit (Roche) according to the manufacturer's instructions.

Preparation of fibrils from recombinant prion protein

25 μM of unlabeled bovine prion protein in PBS containing 0.2 % SDS was incubated for 10 min at room temperature, followed by a twentyfold dilution with PBS. For fibril formation, the diluted reaction mixture was incubated for 48 h at room temperature [14].

PrP fibril formation in serum or plasma

Recombinant FITC-labeled bovine prion protein was incubated in 150 μl serum or plasma at a concentration of 5 or 10 nM for 5–10 min. at 20°C, shaking at 550 rpm in an Eppendorf thermomixer, followed by an increase of the temperature to 37°C h at constant shaking speed. The incubation was continued for 20 h. Samples were then analyzed by flow cytometry.

Flow cytometry

Analysis of the samples was carried out on a FACSVantage flow cytometer (BD Biosciences) at room temperature, measurement time was 30 sec per sample.

Authors' contributions

LT participated in the design of the study, carried out the measurements and drafted the manuscript. ANS participated in the analysis of the data. EM prepared the recom-

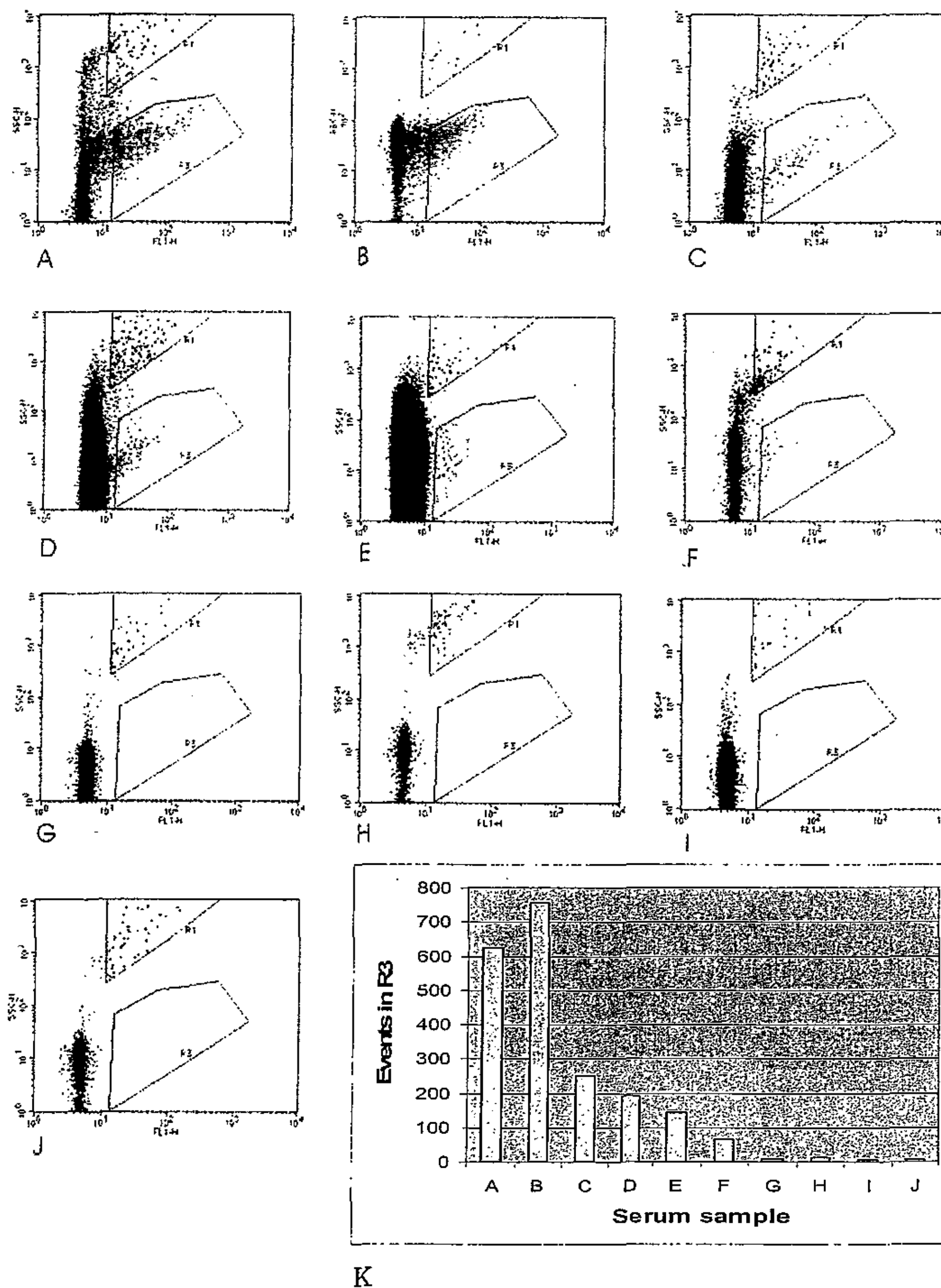

Figure 3

Analysis of serum from BSE-positive cattle. FITC-labeled recombinant prion protein (10 nM) was incubated in 150 μl of the serum samples as described in the methods section and analyzed by flow cytometry. The measurements are shown in a Fluorescence 1 (FL1-H) vs. Side-Scatter (SSC) dot-plot. All six BSE-samples (A-F) can be differentiated from the controls (G-J) by a population of events in region R3 (green dots). Panel K: Quantification of measurements shown in panels A-J.

binant protein. KK and ST were also involved in protein expression and purification. HS participated in the design and coordination of the study. GB conceived of the study and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This work was kindly supported by grant No. 0312711A from the BMBF (Bundesministerium für Bildung und Forschung) in the context of the German National TSE Research Platform. The authors gratefully acknowledge the help of the TSE Research Platform, Munich, and the BFAV Riems, Germany, with respect to the kind gift of biological material in the context of this study.

References

1. Prusiner SB: **Novel proteinaceous infectious particles cause scrapie.** *Science* 1982, **216**:136-144.
2. Kretzschmar HA, Prusiner SB, Stowring LE, DeArmond SJ: **Scrapie prion proteins are synthesized in neurons.** *Am J Pathol* 1986, **122**:1-5.
3. Cashman NR, Loertscher R, Nalbantoglu J, Shaw I, Kascsak RJ, Bolton DC, Bendheim PE: **Cellular isoform of the scrapie agent protein participates in lymphocyte activation.** *Cell* 1990, **61**:185-192.
4. Manson J, West JD, Thomson V, McBride P, Kaufman MH, Hope J: **The prion protein gene: a role in mouse embryogenesis?** *Development* 1992, **115**:117-122.
5. Pan KM, Baldwin M, Nguyen J, Gasset M, Serban A, Groth D, Mehlhorn I, Huang Z, Fletterick RJ, Cohen FE, Prusiner SB: **Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins.** *Proc Natl Acad Sci U S A* 1993, **90**:10962-10966.
6. Caughey BW, Dong A, Bhat KS, Ernst D, Hayes SF, Caughey WS: **Secondary structure analysis of the scrapie-associated protein PrP 27–30 in water by infrared spectroscopy.** *Biochemistry* 1991, **30**:7672-7680.
7. Prusiner SB, Groth DF, Bolton DC, Kent SB, Hood LE: **Purification and structural studies of a major scrapie prion protein.** *Cell* 1984, **38**:127-134.
8. Prusiner SB, McKinley MP, Bowman KA, Bolton DC, Bendheim PE, Groth DF, Glenner GG: **Scrapie prions aggregate to form amyloid-like birefringent rods.** *Cell* 1983, **35**:349-358.
9. Prusiner SB: **Prions.** *Proc Natl Acad Sci U S A* 1998, **95**:13363-13383.
10. Kretzschmar HA, Ironside JW, DeArmond SJ, Tateishi J: **Diagnostic criteria for sporadic Creutzfeldt-Jakob disease.** *Arch Neurol* 1996, **53**:913-920.
11. Brown P, Cervenakova L, Diringer H: **Blood infectivity and the prospects for a diagnostic screening test in Creutzfeldt-Jakob disease.** *J Lab Clin Med* 2001, **137**:5-13.
12. Proske D, Gilch S, Wopfner F, Schätzl HM, Winnacker EL, Famulok M: **ion-protein-specific aptamer reduces PrP^{Sc} formation.** *Chembiochem* 2002, **3**:717-725.
13. Gilch S, Wopfner F, Renner-Muller I, Kremmer E, Bauer C, Wolf E, Brem G, Groschup MH, Schätzl HM: **Polyclonal anti-PrP auto-antibodies induced with dimeric PrP interfere efficiently with PrP^{Sc} propagation in prion-infected cells.** *J Biol Chem* 2003, **278**:18524-18531.
14. Post K, Pitschke M, Schafer O, Wille H, Appel TR, Kirsch D, Mehlhorn I, Serban H, Prusiner SB, Riesner D: **Rapid acquisition of beta-sheet structure in the prion protein prior to multimer formation.** *Biol Chem* 1998, **379**:1307-1317.
15. Pitschke M, Prior R, Haupt M, Riesner D: **Detection of single amyloid beta-protein aggregates in the cerebrospinal fluid of Alzheimer's patients by fluorescence correlation spectroscopy.** *Nat Med* 1998, **4**:832-834.
16. Llewelyn CA, Hewitt PE, Knight RS, Amar K, Cousens S, Mackenzie J, Will RG: **Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion.** *Lancet* 2004, **363**:417-421.
17. Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW: **Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient.** *Lancet* 2004, **364**:527-529.
18. Hunter N, Foster J, Chong A, McCutcheon S, Parnham D, Eaton S, MacKenzie C, Houston F: **Transmission of prion diseases by blood transfusion.** *J Gen Virol* 2002, **83**:2897-2905.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp