Vero E6 cells. A cytopathic effect was observed only in the first passages; thereafter, morphologic characteristics did not differ between infected and control cells. Indirect immunofluorescence assays with the use of polyclonal antibodies against arenaviruses and LCMV showed cytoplasmic distribution of viral antigen. Immunostaining of viral antigens was also seen in infected cells by means of an indirect immunoalkaline phosphatase technique (Fig. 2A). Quantitative RT-PCR assays showed increasing concentrations of viral nucleic acid with serial passage. Examination of infected Vero E6 cells by means of thin-section electron microscopy revealed extracellular particles with morphologic features that are characteristic of arenaviruses (Fig. 2B).

Immunofluorescence assays for serum antibodies that are reactive with infected Vero E6 cells revealed virus-specific IgM and IgG antibodies in the donor that were consistent with acute infection. Plasma and serum specimens from Recipient 2 that had been collected at two time points 19 days apart (11 days and 30 days after transplantation) were available for analysis. Virus-specific IgG and IgM antibodies were detectable only at the second time point, consistent with seroconversion.

Immunohistochemical analysis of specimens of the liver (Fig. 3A) and kidney (Fig. 3B) obtained from Recipient 1 showed focal immunostaining of arenavirus antigens. PCR surveys of 100 archived serum or plasma specimens from solid-organ transplant recipients who were not linked to the cluster and who had undergone transplantation in the same city and during the same time period revealed no evidence of infection with this pathogen.

The 3301-nucleotide S-segment and 7215-nucleotide L-segment sequences were cloned from the kidney of Recipient 1 by means of PCR and sequenced. Phylogenetic characterization was limited by the paucity of available sequences deposited in public databases; nonetheless, L- and S-segment analyses were consistent with the presence of a new arenavirus. Whereas sequences in the nucleoprotein and glycoprotein genes on the S segment were closest to the LCMV strain LE[13] and M1 and M2 isolates[14] (Table 2, and Fig. 1b and 1c of the Supplementary Appendix), the L-segment sequence indicated a closer relationship to Kodoko virus. Strain LE was isolated in France from an infected fetus. M1 and M2 were isolated in Japan from wild mice. Kodoko virus was recently isolated in Africa from wild mice (Fig. 1a of the Supplementary Appendix).[15] Reassortment is well described in arenaviruses and could account for differences in phylogenetic relationships based on L- and S-segment sequences. However, reassortment cannot be implicated without a complete genomic sequence for the viruses used in these phylogenetic analyses.

Table 2. Nucleotide and Amino Acid Homologies of the New Arenavirus to Other Arenaviruses.*

Gene	Accession No.	LCMV Strain	Homology	
			Amino Acid	Nucleotide
			percent	
GPC	AB261990	M2	94	86
NP	AB261990	M2	97	87
L	DQ286932	Marseille 12	82	79
Z	DQ286932	Marseille 12	79	72

* LCMV denotes lymphocytic choriomeningitis virus.

DISCUSSION

Two clusters of transmission of arenavirus through solid-organ transplantation have been reported.[4] In each cluster, recipients linked to a single donor died of an unexplained infectious disease 9 to 76 days after transplantation. In neither cluster did the donor have a history of acute infectious disease or evidence of infection by PCR or serologic analysis; however, in one cluster, a pet hamster that had recently been introduced into the donor's household was found to be infected with the same virus that was detected in the recipients. LCMV was implicated after the results of viral culture and electron microscopy triggered specific immunohistochemical and molecular tests for arenaviruses.

In our cluster, a new arenavirus was first detected through unbiased high-throughput sequencing. Thereafter, the infection was confirmed by means of culture, electron microscopy, and specific immunohistochemical and serologic tests. As in the other two reported clusters of transplant-associated transmission, we detected no viral nucleic acids in the donor and found no history of acute infectious disease; however, the presence of IgG and IgM antibodies confirmed recent infection. We were also unable to obtain any infor-

Table 3. Viral RNA and Antibody Titers in the Donor and Recipients.*

Specimen	Interval between Transplantation and Collection of Specimens	Viral RNA	Antibody Titer
	days	*copies/ml of RNA extract*	
Donor			
Serum	0	ND	1:80 IgG, 1:20 IgM
Spleen	0	ND	NA
Pancreas	0	ND	NA
Recipient 1 (kidney transplant)			
Plasma	0	ND	<1:10 IgG, <1:10 IgM
Plasma	27	889,200	NP
Plasma	33	614,900	NP
Cerebrospinal fluid	33	5,500	NP
Plasma	35†	1,000,000	NP
Urine	35†	88,000,000	NA
Heart	35†	33,200	NA
Spleen	35†	52,600	NA
Liver	35†	2,362,800	NA
Lung	35†	498,600	NA
Cerebrospinal fluid	35†	63,700	NP
Serum	35†	1,440,400	<1:10 IgG, <1:10 IgM
Brain	35†	16,600	NA
Rectal swab	35†	623,200	NA
Nasal swab	35†	55,400	NA
Axillary swab	35†	ND	NA
Kidney	35†	85,900	NA
Recipient 2 (liver transplant)			
Plasma	12	121,900	<1:10 IgG, <1:10 IgM
Mouth swab	24	457,000	NA
Bronchoalveolar lavage	19	1,163,400	NA
Cerebrospinal fluid	24	ND	NP
Plasma	24	346,200	NP
Serum	31†	347,600	1:40 IgG, 1:20 IgM
Recipient 3 (kidney transplant)			
Serum	−235	ND	<1:10 IgG, <1:10 IgM
Serum	0	ND	NP
Serum	24	415,500	NP
Serum	28	565,100	<1:10 IgG, <1:10 IgM

* NA denotes not applicable, ND not detected, and NP not performed.
† Specimens were obtained after death.

mation indicating that the donor had been exposed to rodents; however, his history of recent travel suggests that he may have been infected before returning to Australia from southern Europe, where such exposure may have occurred in a rural area.

Although we have not fulfilled Koch's postulates, evidence implicating this new virus in the

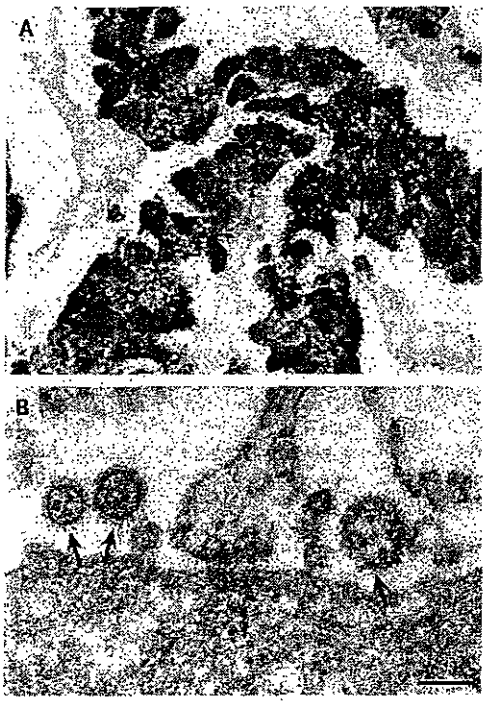

Figure 2. Propagation of the New Arenavirus in Tissue Culture.

Panel A shows immunostaining of viral antigens in infected cells by means of an indirect immunoalkaline phosphatase technique. Panel B shows an electron micrograph of extracellular arenavirus-like virions. Particles (arrows) are round, vary in size, and have surface projections on the perimeter. Cellular ribosomes are visible within the virions. The length of the bar corresponds to 100 nm.

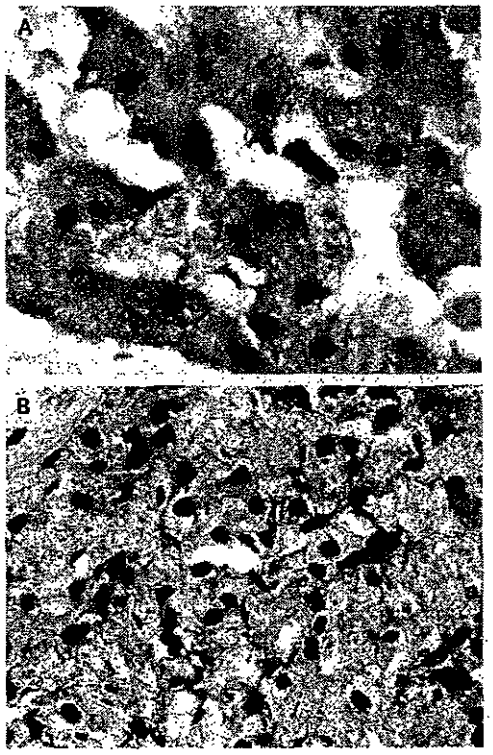

Figure 3. Predominantly Membranous Distribution of Arenavirus Antigen.

The distribution of the arenavirus antigen is shown in the liver (Panel A) and kidney (Panel B) of Recipient 1. Formalin-fixed, paraffin-embedded tissue sections were incubated with polyclonal rabbit antiserum against lymphocytic choriomeningitis virus followed by alkaline phosphatase–conjugated secondary antibodies against rabbit IgG.

outbreak of infection among patients who received transplants is compelling. All three recipients received organs from the same donor and died within days of one another after febrile illness. Identical viral sequences were obtained from all the recipients. The virus is new and was not detected in 100 organ recipients who were not linked to this cluster. The results of serologic analysis of specimens obtained from the donor were consistent with recent infection, and seroconversion was observed in one recipient.

Unbiased high-throughput sequencing has been used to characterize complex mixtures of microflora in environmental contexts[16]; we have shown that this strategy can be used to address a suspected outbreak of infectious disease. Its use in the context of investigating a cluster of cases of acute disease associated with organ transplantation facilitated the rapid implication of a new arenavirus not detected by other methods. This technique may prove useful as a new tool in the identification and surveillance of pathogens in chronic as well as acute disease.

Supported by grants from the National Institutes of Health (U54AI57158, to the Northeast Biodefense Center–Lipkin; U01AI070411; AI062705; and HL083850).

Drs. Du, Simons, and Egholm report being employees of 454 Life Sciences. Dr. Lipkin reports being a member of the scientific advisory board of 454 Life Sciences during a portion of the time the work reported here was pursued. Drs. Du, Simons, Egholm, and Lipkin report holding stock options in 454 Life Sciences before it was purchased by Roche Diagnostics in May 2007. No other potential conflict of interest relevant to this article was reported.

We thank David Riches for skillful technical assistance.

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別紙様式第2-1

No. 26

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 10. 22	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)		岡田義昭, 水澤左衛子. 2007年プリオン研究会	日本

研究報告の概要

○BSE由来プリオンのin vitro感染系の確立とその応用

目的:輸血によるvCJD感染が英国で4例報告され、分画製剤を含めた血液製剤の安全性確保が重大な課題になっている。しかし、除去評価の対象となる検体や除去効果を評価する適当な方法がないのが現状である。我々は血液を対象としているため、さらに血液中にどのような様式でPrP^{res}が存在するのか、明らかにする必要がある。そこで我々は、BSE感染ウシ由来の脳乳剤を用いてPrP^{res}のin vitro感染系の確立を試みたので報告する。

方法:BSE感染ウシ由来の脳乳剤をマウス及びヒト由来の神経系細胞株、及び血液由来細胞株に添加し、継代しながら経時的にウエスタンブロット法(WB)を行いPrP^{res}の有無を検討した。PrP^{res}は、核成分を除いた細胞溶解液をPK20 μg/mL、37℃、1時間処理後、メタノール沈殿によって抽出し、ウサギ抗プリオン抗体を用いたWBにて検出した。また、継代した感染細胞の培養上清を段階希釈し、非感染細胞に感染させ、PrP^{res}が伝達されるか検討した。さらに、20nmのウイルス除去膜を用いてPrP^{res}の除去が可能か検討した。

結果:ヒト由来グリオーマ細胞株から30kD付近にPK耐性で抗プリオン抗体に反応する2本のバンドが検出された。このバンドは非感染細胞には存在しなかった。30kD付近の2本のバンドは感染後10週前後からWBによって検出可能になり、14週頃まで検出された(細胞によっては20～25週頃まで検出することもできた)。また、9ヵ月継代した感染細胞の培養上清を非感染細胞に感染させたところ、30kD付近にPK耐性の2本のバンドが検出され、伝達性があることが明らかになった。さらに20nmのウイルス除去膜を用いて培養上清を濾過したところ、無処理に比較して感染価は約5Log減少し、ウイルス除去膜によって伝達性が減少することが認められた。

考察:BSE由来のPrP^{res}を感染させた細胞から抗プリオン抗体に反応する約30kDのPK耐性のバンドが検出された。培養上清によって同様のバンドが非感染細胞に伝達されたことから、脳乳剤から検出されるPrP^{res}とはバンドのパターンは異なるもののin vitroにおいてBSEの感染が成立したと考えられた。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
BSE感染ウシ由来の脳乳剤を用いたPrP^{res}のin vitro感染系の確立を試みたところ、9ヵ月継代した感染細胞の培養上清に伝達性があることが明らかになった。また、20nmのウイルス除去膜によって培養上清の伝達性が減少することが認められたとの報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。

(5)

JRC2007T-080

BSE 由来プリオンの in vitro 感染系の確立とその応用

○岡田義昭、水澤左衛子　　国立感染症研究所　血液・安全性研究部

（目的）輸血によるｖＣＪＤ（variant Creutzfelt Jacob Disease）感染が英国で 4 例報告され、分画製剤を含めた血液製剤の安全性確保が重大な課題になっている。しかし、除去評価の対象となる検体や除去効果を評価する適当な方法がないのが現状である。我々は血液を対象としているため、さらに血液中にどのような様式で PrP^{res} が存在するのか、明らかにする必要がある。そこで我々は、BSE 感染ウシ由来の脳乳剤を用いて PrP^{res} の in vitro 感染系の確立を試みたので報告する。

（方法）BSE 感染ウシ由来の脳乳剤をマウス及びヒト由来の神経系細胞株、及び血液由来細胞株に添加し、継代しながら経時的 にウエスタンブロット法（以下ＷＢ）を行い PrP^{res} の有無を検討した。PrP^{res} は、核成分を除いた細胞溶解液をＰＫ20μg/mL、37℃、1 時間処理後、メタノール沈殿によって抽出し、ウサギ抗プリオン抗体を用いたＷＢにて検出した。また、継代した感染細胞の培養上清を段階希釈し、非感染細胞に感染させ、PrP^{res} が伝達されるか検討した。さらに、20ｎｍのウイルス除去膜を用いて PrP^{res} の除去が可能か検討した。

（結果）ヒト由来グリオーマ細胞株から 30Ｋｄ付近に PK 耐性で抗プリオン抗体に反応する 2 本のバンドが検出された。このバンドは非感染細胞には存在しなかった。３０Ｋｄ付近の 2 本のバンドは感染後 10 週前後からＷＢによって検出可能になり、14 週頃まで検出された（細胞によっては 20～25 週頃まで検出することもできた）。また、9 ヶ月継代した感染細胞の培養上清を非感染細胞に感染させたところ、30Ｋｄ付近に PK 耐性の 2 本のバンドが検出され、伝達性があることが明らかになった。さらに 20ｎｍのウイルス除去膜を用いて培養上清を濾過したところ、無処理に比較して感染価は 約 5Log 減少し、ウイルス除去膜によって伝達性が減少することが認められた。

（考察）BSE 由来の PrP^{res} を感染させた細胞から抗プリオン抗体に反応する約 30Ｋの ＰＫ耐性のバンドが検出された。培養上清によって同様のバンドが非感染細胞に伝達されたことから、脳乳剤から検出される PrP^{res} とはバンドのパターンは異なるものの in vitro において BSE の感染が成立したと考えられた。

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 9. 20	該当なし	

一般的名称	抗HBs人免疫グロブリン	研究報告の公表状況		公表国
販売名(企業名)	抗HBs人免疫グロブリン「日赤」(日本赤十字社)		Foster P. Prion 2007; 2007 Sep 26-28; Edinburgh.	英国

研究報告の概要

○vCJDはヒト血漿製剤によって伝播したのか？20年間と集計
vCJDと診断された人の血漿が、診断以前に英国国立健康増進局(NHS)によって血液製剤の製造に使用されていたため、予防措置として、英国の供血者由来の血漿からの血漿由来製剤の製造中止が1998年に決定された。これ以来、血漿分画製剤は、NHSによって米国及びヨーロッパから購入した血漿を使用して製造されるか、営利企業から直接購入されてきた。
後にvCJDと診断された11人の供血者由来の供血が、1987年6月から1998年9月にかけて出荷された175バッチの様々な血漿分画製剤の原料に含まれていたということが知られている。最初の製品出荷から20年が経過したにもかかわらず、これらの製剤に関連したvCJD症例は発生していない。このことは、赤血球によるvCJD伝播の可能性を示す症例が、輸血後6.5年、7.8年、8.3年で発症したことと対照的である。
血漿分画製剤による明らかな伝播が見られないことについては幾つかの説明がなされている。
(1)供血された血漿中にプリオンの感染性がない
(2)供血された血漿中にプリオンの感染性はあるが、製造工程の希釈や感染性の低減によって、製品には感染性がない
(3)製品にプリオンの感染性はあるが、潜伏期間が長いあるいは投与された患者に感受性がないため、まだ発症していない
プリオン除去の範囲を特定するためスコットランド輸血サービスで血漿分画製剤の製造に用いられている方法を検討した。これらの実験は、プリオン除去能は全体として、中間純度の第Ⅷ因子濃縮製剤で2.7log、中間純度の第Ⅸ因子濃縮製剤で3.0log、トロンビンで5.8log、フィブリノゲンで6.2log以上、免疫グロブリンで6.5log以上、高純度の第Ⅸ因子製剤で7.4log、アルブミンで11.5log以上だった。

報告企業の意見	今後の対応
1987年6月から1998年9月にかけて英国で出荷された血漿分画製剤の原料には、後にvCJDと診断された11人の供血者由来の血漿が含まれていたが、製剤に関連したvCJD症例は発生しておらず、製剤によってばらつきはあるものの製造工程にはプリオン除去効果があるとの報告である。	異常プリオンが本製剤の製造工程で効果的に除去されるとの実験成績を疫学的に裏付けた報告と言える。しかし、輸血によるvCJDに感染が示唆されていることから、今後も情報の収集に努める。尚、日本赤十字社はvCJD他の血液を介する感染防止の目的から、輸血歴のあるドナー、および英国を含む欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。特に英国については、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より1980年～1996年に1日以上の英国滞在歴のある方からの献血を制限している。

使用上の注意記載状況・その他参考事項等

抗HBs人免疫グロブリン「日赤」

血液を原料とすることに由来する感染症伝播等
vCJD等の伝播のリスク

Epidemiology, Risk Assessment and Transmission

P04.101

Development of a Standardised Approach to Assess the Effectiveness of Current and New Decontamination Technologies against TSE Agents

Hesp, IJR; Kirby, E; Dickinson, J; Dennis, M; Cornwall, M; Raven, NDH; Sutton, JM
Health Protection Agency, Research, UK

Background: The development of inactivation methods for Transmissible spongiform encephalopathies (TSEs) is an urgent requirement in relation to the potential for iatrogenic transmission of variant Creutzfeldt Jakob Disease (vCJD). The evaluation of the effectiveness of such methodologies requires a highly sensitive and specific assay or a combination of assays. With current cellular and biochemical based assays still in development, the bioassay remains the accepted approach to assess effectiveness; however, careful matching between the TSE strain and host species is required to help ensure that the risks are appropriately evaluated with regard to vCJD transmission.

Aims: The project aims to develop a robust system to assess proposed TSE inactivation technologies focusing on a model using the TSE strain, BSE-301V, designed to mimic the key features of possible vCJD transmission via contaminated *surgical instruments. The dynamic range of the model was determined using a titration* series of infectivity which in the first instance was 'tested' using a conventional autoclave based process.

Methods. BSE-301V infected mouse brain homogenate, previously titrated to $x10^9$ ID_{50} per gram, was dried onto the surface of surgical steel suture wires using a standardised process. Wires were implanted i.c. into VM mice and monitored for clinical symptoms for up to 550 days.

Results: For the wire-based titration series clinical symptoms were observed in animals from groups across a 6-log dilution range, however, at dilutions below 10^{-3} transmission rates fell below 50%, suggesting that the useful range is around 4-logs. Data will be presented comparing the surface bound titration results with the equivalent in-solution titration series. The ongoing results from the decontamination studies will also be presented in relation to the titration data generated.

Conclusions: Methods have been established to ensure a consistent exposure of wires to the decontamination process with no further manipulations of the carriers post processing. Using this protocol a titration series has been established for BSE-301V on surgical steel that potentially covers a 4-log range. The use of these protocols to evaluate novel prion decontamination methods will be discussed.

P04.102

Has vCJD been Transmitted by Human Blood Plasma Products? 20 Years and Counting

Foster, P
Scottish National Blood Transfusion Service, Protein Fractionation Centre, UK

The diagnosis of vCJD in a patient whose plasma had previously been used in the preparation of blood plasma products by the NHS led to the decision in 1998 that the preparation of plasma derivatives from UK-donor plasma should cease as a precautionary measure. Since then, plasma products have either been manufactured by the NHS, using plasma purchased from the USA and Europe, or purchased directly from commercial companies.

It is now known that donations from 11 individuals, later diagnosed with vCJD, had been included in the preparation of a total of 175 batches of different plasma products that were released for use between June 1987 and September 1998. No cases of vCJD have been associated with these products, although 20 years have elapsed since the first implicated batches were released for use. This contrasts with 3 instances of *probable transmission of vCJD by red cells in which symptoms of vCJD developed in* recipients 6.5 years, 7.8 years and 8.3 years after transfusion.

There are a number of possible explanations for the apparent absence of transmision by plasma products.

(1) Prion infectivity was not present in the donated plasma.
(2) Prion infectivity was present in the donated plasma but not in the manufactured products, due to dilution or removal of infectivity by the manufacturing process.
(3) Prion infectivity was present in manufactured product(s) but has not resulted in clinical symptoms of vCJD because of either a prolonged incubation period or a lack of suceptibility in recipients.
The methods used for the manufacture of blood plasma products by the Scottish National Blood Transfusion Service have been examined to determine the extent to which removal of prions might have occurred. These experiments indicate a possible overall prion reduction of 2.7 logs for intermediate-purity factor VIII concentrate (Z8), 3.0 logs for intermediate-purity factor IX concentrate (DEFIX), 5.8 logs for thrombin, ≥6.2 logs for fibrinogen, ≥6.5 logs for immunoglobulin, 7.4 logs for high-purity factor IX concentrate and ≥11.5 logs for albumin.

P04.103

Femtograms-Detection of PrPSc in Biological Samples using Chemically Synthesized RNA-Aptamer

Nagata, T[1]; Yokoyama, T[2]; Sekiya, S[3]; Nishikawa, S[3]; Noda, K[1]
[1]National Veterinary Assay Laboratory, Japan; [2]National Institute for Animal Health, Japan; [3]National Institute of Advanced Industrial Science and Technology (AIST), Japan

For the safety of biological products, it is one of our major concerns to reduce the TSE-risk of cattle-blood derived materials such as serum and plasma. For the detection of possibly contaminated abnormal isoform of prion protein (PrPSc) in the biological samples, it is indispensable to develop a highly sensitive PrP detection procedure. Here, we have developed an aptamer-beads PrP-concentration procedure by using RNA-aptamer 60-3 which binds to recombinant mouse PrP with high affinity (Kd = 5.6 nM) (1).

The RNA-aptamer 60-3 was chemically synthesized employing a novel RNA synthetic method with a 2'-O-(2-cyanoethoxymethyl) protecting group (2), with 2'OMe-pyrimidine modification for RNase resistance, and conjugated with biotin. The aptamer was then bound to streptavidin-coated magnetic beads (60-3 aptamer-beads) and used for pull-down assays. The pulled-down PrPSc was analyzed by Western blotting.

The 60-3 aptamer-beads demonstrated the enrichment of PrPSc from the 20-million times diluted scrapie-infected mouse brain (50ml of 50ng brain equivalent /ml). Comparing to phosphotungstic acid (PTA) concentration method, the 60-3 aptamer-beads revealed more than 100 times efficiency in concentrating PrPSc spiked in bovine serum. Moreover, the 60-3 aptamer-beads showed binding ability to PrPSc in highly diluted BSE-infected bovine brain.

The present Aptamer-beads pull-down procedure enables us to perform a femtograms-detection of PrP. The procedure was also proven to be applicable to BSE-PrPSc. The present aptamer-beads system could serve as a resource for prion-removal column and serum prion assays, and potentially achieve the safety of the blood derived biological products.

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P04.104

Survival of Prion Proteins in Environmental Matrices

Maluquer de Motes, C[1]; Torres, JM[2]; Pumarola, M[3]; Girones, R[1]
[1]University of Barcelona, Spain; [2]Centro de Investigación en Sanidad Animal, Spain; [3]Autonomous University of Barcelona, Spain

Several publications have suggested the environment as a possible route of transmission, especially for sheep scrapie and cervid Chronic Wasting Disease (CWD). The role of the environment as a reservoir for these disorders is difficult to prove and faces a considerable lack of information. In this work, different methodologies have been developed to evaluate the survival and inactivation of TSE agents in environmental matrices.

Different slaughterhouse and urban sewage samples were spiked with diverse strains of either scrapie or BSE agents and kept under controlled conditions for extended periods of time. Aliquots of every experiment were sequentially collected and concentrated according to a methodology specifically selected for each type of matrix. Sensitivity of the methods developed was estimated among 2-10 µg of infected tissue. PrPres was finally detected by western blot. Films were then transformed into digital pictures, signal intensities were quantified and regression models were computed.

According to the results obtained, scrapie agent showed higher stability than BSE in all the environments studied. However, no significant differences were observed among mouse-passaged scrapie strains and sheep scrapie. The regression models provided t90 and t99 values (times of incubation necessaries for 90% and 99% reduction of PrPres levels). In urban sewage, i.e., t99 was estimated as about 50 and 22 days for scrapie and BSE respectively. In general, the effect of the matrix was clearly observed in all the experiments, showing up to a 6-8 fold higher reduction of PrPres levels in comparison to PBS controls.

As some of the inocula were titrated in terms of infectious doses, we approximated the decay of PrPres levels to the reduction of infectivity for both agents. In slaughterhouse wastewater, i.e., two-log reduction was observed for both agents after 30-35 days of incubation. Data on infectivity will be confirmed by a series of bioassay experiments.