Macaque model of variant Creutzfeldt-Jakob disease infection

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Abstract

Background

Variant Creutzfeldt-Jakob disease (vCJD) is a fatal neurodegenerative disease of humans infected with the agent of bovine spongiform encephalopathy (BSE). vCJD and BSE are transmissible spongiform encephalopathies (TSEs, prion diseases). Blood transfusions have transmitted vCJD in rare cases. These transmissions might have been prevented had a vCJD blood donor screening test been available.

Goal

The goal of this project was to demonstrate the utility of CBER macaque vCJD blood materials to develop and validate vCJD assays.

Experimental design

We experimentally infected macaques with vCJD agent and collected blood at intervals during incubation and clinical periods. We assayed the blood samples for the disease biomarker PrP^{TSE} using a test called protein misfolding cyclic amplification (PMCA). We inoculated aliquots of blood collected during the final stage of illness into vCJD-susceptible mice to confirm that vCJD-infected macaques, like humans, harbored the infectious agent in their blood.

Results and conclusions

We detected PrP^{TSE} in all aliquots of macaque blood starting from earliest time points suggesting that PrP^{TSE} could be used as a preclinical disease marker in blood screening tests. We assayed brains of mice inoculated with macaque blood for PrP^{TSE} as indicator of transmission of infectivity to mice. We detected PrP^{TSE} in few mouse brains, confirming vCJD infectivity in macaque blood. This work showed relevance of the macaque model to study vCJD agent in blood and demonstrated that our macaque blood samples provide suitable surrogate reference materials to validate vCJD blood tests.

Introduction

Validation of a test to detect abnormal prion protein (PrP^{TSE}, marker of all TSEs) in infected blood would require blood reference materials. To this end, we prepared blood samples from vCJD-infected macaques as surrogates for infected human blood which is not available in sufficient quantities. These materials were proposed as candidate reference materials to be used by qualified investigators to develop and validate vCJD blood donor screening assays. In 2020, we shared a portion of our macaque blood inventory with UK NIBSC to distribute outside the US.

As part of the required characterization of the macaque blood candidate reference materials, we needed to show that those samples contained vCJD infectivity and that PrP^{TSE} could be detected. PrP^{TSE} is the target of all in vitro assays under development.

We used transgenic mice overexpressing normal bovine prion protein (TgBo110) to demonstrate infectivity. We previously confirmed that these mice were highly susceptible to brain-derived vCJD infectivity (Bett et al., *JGV*, 2018) but their sensitivity to blood infectivity was unknown. We report here the results of those studies.

To detect PrP^{TSE}, we used PMCA. PMCA increases the concentration of PrP^{TSE} in vitro. In our modified PMCA, brain homogenate from normal red-backed voles (expressing the vole prion protein with serine/serine at codon 170) was mixed with an aliquot of processed whole blood from an infected macaque (Nemecek et al., *PLoSOne*, 2013, McDowell et al., *Transfusion*, 2015). Samples were subjected to cycles of incubation and sonication during which PrP^{TSE}, present in the macaque blood, converted normal vole prion protein into new PrP^{TSE}.

Materials and Methods

The White Oak Animal Care and Use Committee reviewed and approved all animal studies.

Macaque blood

We used 10% macaque brain homogenate infected with vCJD to inoculate 3 macaques intraperitoneally and intravenously. We began to draw blood samples periodically starting 2 months post-inoculation (mpi) and continuing every 1 or 2 months until the end of the study. Blood was collected in either citrate or EDTA anticoagulants and processed immediately. Ten percent of the total volume of blood collected was removed and the remaining was separated into plasma, buffy coat, and RBCs.

Mouse inoculations

TgBo110 mice were bred in the White Oak vivarium. Aliquots of buffy coat from 3 infected macaques were inoculated intracerebrally into a group of mice (30µl/mouse). Approximately, 5ml of whole blood were processed to concentrate PrP^{TSE} and mice were inoculated with resuspended pellets. Mice were monitored for 2 years for signs of vCJD. All mice were necropsied and a portion of each brain was removed for biochemical confirmation of PrP^{TSE} using a commercial veterinary test: IDEXX Herdchek BSE-Scrapie Antigen Test kit (IDEXX test).

PMCA

We concentrated PrP^{TSE} from 250µl of macaque whole blood by ultra-centrifugation. Next, we resuspended the blood pellets in 100µl of 10% normal vole brain homogenate (VBH) and conducted PMCA. For PMCA with mouse brains, we used 25µl of 10% TgBo110 mouse brain homogenate in 75µl of VBH. PrP^{TSE} was amplified thorough repeated cycles of sonication and incubation. 1 cycle of sonication: 10 sec. pulse 15 min incubation at 37°C.1st round of sonication – 288 cycles, 72 hours duration. After 1st round of sonication, samples were diluted 1:10 with fresh substrate and subjected to 2nd round of sonication. Aliquots were removed for Western blot test.

Generation of infected macaque blood

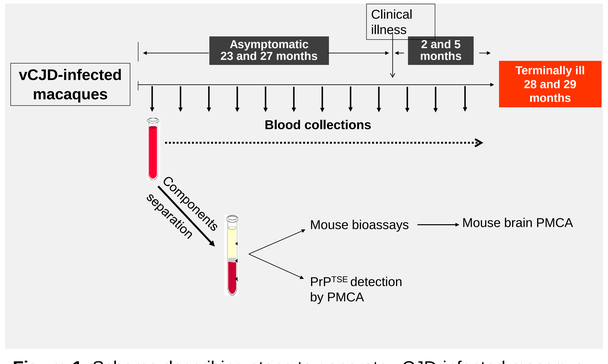

Figure 1. Scheme describing steps to generate vCJD-infected macaque blood components. Blood was collected throughout the incubation period and overt (clinical) illness. Only blood components at terminal phase were assayed by mouse bioassays. However, aliquots from almost every blood collection were tested by PMCA (Figure 2).

Results and Discussion

Detection of PrP^{TSE} in macaque blood

We used PMCA to detect PrP^{TSE} in macaque blood. Among all blood components tested, we found whole blood to be the most sensitive and reproducible test material for PMCA. The results in Figure 2 demonstrate that PrP^{TSE} was detectable starting at the 2nd month post-inoculation and remained detectable throughout aymptomatic infection and during clinical illness. Variability of signals observed in the figure does not reflect fluctuations in PrP^{TSE} concentration but rather the non-quantitative nature of Western blots. Assuming that PrP^{TSE} is a surrogate for infectivity, our results indicate that infectivity was present in the macaques long before clinical signs appeared, consistent with human transmissions of vCJD by blood obtained from apparently healthy donors who only later became ill with vCJD.

Detection of infectivity in macaque blood

Detecting infectivity requires animal bioassay. TgBo110 mice show mild clinical signs not easy to recognize. Thus, we relied exclusively on detecting PrP^{TSE} in brains of the inoculated animals to assign infected/not-infected status. (PrP^{TSE} is present only in infected mouse brains.) Our standard PrP^{TSE} assay was a veterinary diagnostic test (IDEXX) previously showed to be more sensitive than Western blot (Gregori et al., *Transplantation*, 2017). Table 1 shows 3 mice inoculated with 2 blood inocula had IDEXX-positive brains, indicating extremely low transmission rates of macaque blood infectivity.

PMCA mouse brain homogenates. Next, we increased sensitivity of PrP^{TSE} detection in mouse brains using PMCA. The same 3 mice were again positive by PMCA but we also detected other mice with reproducible PrP^{TSE} signals. PMCA testing of mice inoculated with whole-blood concentrates is not complete. Nevertheless, partial results to date, Table 1, show that buffy coat was a better test material to detect infectivity than whole blood—a result consistent with those from other animal models of TSE infections such as hamsters, mice and sheep. It is also notable that all PMCA-positive mice succumbed to vCJD only after long incubations (> 400 dpi), suggesting that samples contained very low levels of infectivity. Importantly, we detected PrP^{TSE}-positive mice inoculated with terminal blood samples from all 3 macaques including the blood of one macaque collected 1 month before the appearance of clinical signs (preclinical). Using results with PMCA, we calculated attack rates between 8-12%.

PMCA with macaque whole blood

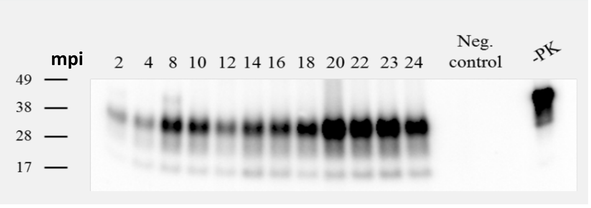

Figure 2. Western blot results from a 2nd round of PMCA with macaque whole blood collected at the indicated months post-infection (mpi). The 3 bands correspond to the 3 glycosylation isoforms of prion protein. All samples except the sample on the right (-PK) were subjected to proteinase K (PK) digestion to remove normal prion protein and detect only abnormal prion protein, not present in normal brain. The control lane showed no signal because normal prion protein present in normal (negative) brain homogenate did not amplify PrP^{TSE} by PMCA. On the left are the molecular weight standards.

PMCA with TgBo110 mouse brains

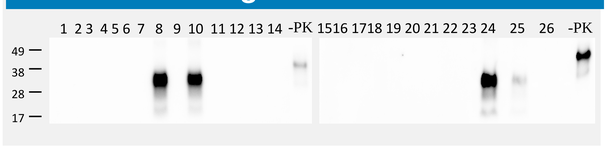

Figure 3. Western blot results after a 5th round of PMCA with 23 10% brains of mice inoculated with buffy coat from a terminal vCJD-infected macaque (lines 1-23). Two out of 23 brains were PrP^{TSE}-positive (lines 8 and 10).
Positive controls: line 24 - 10% vCJD TgBo110 brain homogenate and line 25 – 10^{-6} vCJD macaque brain homogenate. Negative control: line 26 – brain homogenate of a mouse inoculated with normal macaque blood.

Table 1. Summary results of all mice inoculated in the macaque blood infectivity studies. Animals were monitored for 2 years.

Inoculum	brains analyzed/ total	Positive by Western blot or IDEXX	Positive by PMCA	Average DPI for positive
Normal macaque buffy coat	62/62	0	0	-
CO7423 buffy coat (preclinical)	58/58	2(3.4%)	6 (10.3%)	528 ± 109
CO7422 buffy coat	58/58	1 (1.7%)	7 (12%)	678 ± 50
CO16999 buffy coat	51/51	0	4 (7.8%)	751 ± 4
CO7423 buffy coat	53/53	0	5 (9.4%)	626 ± 136
CO16999 whole blood concentrate	10/15 ongoing	0	0	-
CO7422 whole blood concentrate	10/12 ongoing	0	0	-
CO7423 whole blood concentrate	13/15 ongoing	0	1 (7.7%)	547

DPI = days post-inoculation

Conclusions

- Blood from macaques infected with vCJD contains detectable PrP^{TSE}. This is an important finding, because PrP^{TSE} is the target of all TSE tests and, thus, we showed that our macaque blood could be used to develop and validate candidate tests for vCJD. Furthermore, we found that PrP^{TSE} was present in blood when animals were still asymptomatic. This is critical, because a relevant TSE blood test must detect PrP^{TSE} in the preclinical phase of the disease when donors, unaware of their infected status, could donate contaminated blood.
- We demonstrated that macaque blood collected during terminal illness and one month before clinical signs appeared was infectious. This is important because it correlates PMCA-detected PrP^{TSE} to infectivity. It also supports using the macaque model of vCJD infection as a valid surrogate for human blood infectivity. Our studies showed that macaques, like humans, are susceptible to vCJD and have a long asymptomatic incubation phase during which blood is infectious and contains PrP^{TSE}.
- We achieved the main goal of this project: demonstrating the potential relevance of CBER macaque vCJD blood materials to develop and validate future vCJD assays.