Immediate and Ongoing Detection of Prions in the Blood of Hamsters and Deer following Oral, Nasal, or Blood Inoculations

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Infectious prions traverse epithelial barriers to gain access to the circulatory system, yet the temporal parameters of transepithelial transport and persistence in the blood over time remain unknown. We used whole-blood real-time quaking-induced conversion (wbRT-QuIC) to analyze whole blood collected from transmissible spongiform encephalopathy (TSE)-inoculated deer and hamsters throughout the incubation period for the presence of common prion protein-conversion competent amyloid (PrP^C-CCA). We observed PrP^C-CCA in the blood of TSE-inoculated hosts throughout the disease course from minutes postexposure to terminal disease.

Prions have been detected in the blood of infected animals (1–6), including humans (7–10), under a variety of experimental conditions. It is not known when prions enter the blood following exposure to the infectious agent and whether blood-borne prions persist or are cleared during the protracted asymptomatic period of prion disease. To assess the temporal characteristics of prionemia in transmissible spongiform encephalopathy (TSE)-infected hosts, we applied a modified version of real-time quaking-induced conversion (RT-QuIC) (11) for the detection of common prion protein-conversion competent amyloid (PrP^C-CCA) in whole blood samples (12) collected at various time points throughout the incubation period of animals inoculated via several routes of exposure.

White-tailed deer (WTD) and Reeves' muntjac deer (MJD) were sourced from chronic wasting disease (CWD)-free areas—the University of Georgia (Athens, GA) Warnell School of Forestry and Natural Resources or Cervid Solutions, Inc. (Tellico, TN). Syrian hamsters (10- to 11-week-old males) were obtained from Harlan Sprague Dawley (Indianapolis, IN). All animals were maintained in biosafety level 2+ (BSL2+) indoor facilities and housed and cared for as previously described (12).

To maximize animal use, whole blood collected from previous and contemporary studies (Table 1) (5, 12–14) was analyzed to study the full course of disease, from initial TSE exposure through terminal disease. All data were generated from new 1-ml aliquots of blood. Susceptible naive hosts were exposed to TSEs by several routes (Table 1). There were 44 CWD-exposed deer (WTD, $n = 34$; MJD, $n = 10$). Seven of 44 deer underwent intravenous exposure, and the other 37 were exposed by other peripheral routes, including aerosol ($n = 6$) (13), oral ($n = 19$), intranasal ($n = 6$), and oral/subcutaneous ($n = 6$) (14). Fifty-four Syrian hamsters were extranasally (e.n.) exposed to HY-TME, a hyper strain of transmissible mink encephalopathy. A total of 14 naive deer (WTD, $n = 6$; MJD, $n = 8$) and 36 naive hamsters served as negative controls.

Whole-blood samples were collected. For WTD and MJD, 10-ml samples of whole blood each were obtained, heparinized (200 U/ml), and treated with 14% citrate-phosphate-dextrose-adenine (CPDA) at baseline (prior to inoculation), at 15, 30, and 60 min, at 24, 48, and 72 h, at 1 to 30 months postexposure (post-

inoculation [p.i.]), and at terminal clinical disease (17 to 30 months p.i.). For Syrian hamsters, 2-ml samples of whole heparinized blood (200 U/ml) were obtained at baseline, at 15, 30, and 60 min, at 24 and 72 h, at 5, 7, and 10 days, and at 2-week intervals for 20 weeks p.i. This represents 0 to 100% of the TSE disease course.

The blood was analyzed using wbRT-QuIC (specific treatments for whole blood prior to RT-QuIC) (12). In brief, individual whole-blood samples (0.5 ml) underwent 4 freeze-thaw cycles and bead homogenization, followed by sodium phosphotungstate precipitation with NaPTA (4% Sarkosyl, 298 U/ μ l benzoylase, 4% sodium phosphotungstic acid) and centrifugation (14,000 rpm for 30 min). The resulting pellet was resuspended in 50 μ l 0.1% Sarkosyl. The wbRT-QuIC reaction mixtures contained 2 μ l NaPTA-precipitated whole blood in 98 μ l reaction buffer (NaCl, 5 $\times$ phosphate-buffered saline [PBS], EDTA, thioflavin T, and truncated recombinant Syrian hamster PrP [SHrPrP 90-231]) (15, 16). Reactions (8 replicates/sample) were set up in 96-well optic plates, and the plates were placed in a BMG Fluostar fluorescence reader for 62.5 h at 42°C (250 cycles, where 1 cycle is 15 min of 1 min of shaking and 1 min of fluorescence measurement). Assay controls consisted of 10% TSE⁺ deer or hamster brain homogenate (10^{-4} to 10^{-7} in triplicate with 1 $\times$ PBS plus 0.1% Triton X-100) and whole blood (10^{-2} with 8 replicates/sample representing 4 replicates on 2 plates on different days) from deer or hamsters of known TSE status. Sample positivity was determined if 1

Received 20 March 2015 Accepted 26 April 2015

Accepted manuscript posted online 29 April 2015

Citation Elder AM, Henderson DM, Nalls AV, Hoover EA, Kincaid AE, Bartz JC, Mathiason CK. 2015. Immediate and ongoing detection of prions in the blood of hamsters and deer following oral, nasal, or blood inoculations. *J Virol* 89:7421–7424. doi:10.1128/JVI.00760-15.

Editor: S. Perlman

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doi:10.1128/JVI.00760-15

TABLE 1 Cervid and hamster TSE and sham exposure history

Exposure route ^a	Animal(s) (n)	Inoculum ^b	% of disease course when samples were taken	Onset of clinical disease
Cervids				
i.v. transfusion (total, 7/44 [WTD, n = 5; MJD, n = 2])	WTD (4)	0.55 g 10% CWD ⁺ brain homogenate	Baseline to 0.3% (72 h p.i.)	Asymptomatic
	WTD (1) ^c	225 ml CWD ⁺ whole blood	Baseline to 100% (12 mo p.i.)	100% of disease course (12 mo p.i.)
	MJD (2)	20 ml CWD ⁺ CPDA-treated whole blood	Baseline to 0.3% (72 h p.i.)	Asymptomatic
Other peripheral exposures (total, 37/44 [WTD, n = 29; MJD, n = 8])				
Aerosol	WTD (6) ^d	5% (wt/vol) CWD ⁺ brain (2 ml)	Baseline to 100% (25 mo p.i.)	Range, 68 to 100% of disease course (17 to 25 mo p.i.)
p.o.	WTD (19)	10% CWD ⁺ brain (1 g)	Baseline to 100% (30 mo p.i.)	Range, 57 to 100% of disease course (17 to 30 mo p.i.)
p.o. + i.n.	WTD (4)	10% CWD ⁺ brain (0.5 g p.o. + 0.05 g i.n.)	Baseline to 0.3% (72 h p.i.)	Asymptomatic
	MJD (2)	10% CWD ⁺ brain (0.5 g p.o. + 0.05 g i.n.)	Baseline to 0.3% (72 h p.i.)	Asymptomatic
p.o. + s.c.	6 MJD (6) ^e	10% CWD ⁺ brain (0.5 g p.o. + 0.5 g s.c.)	Baseline to 100% (24 mo p.i.)	Range, 75 to 100% of disease course (18 to 24 mo p.i.)
SH (total, 54) e.n.				
	SH (54)	HY-TME ⁺ brain 10 ^{6.8} LD ₅₀ (10 µl total, 0.5 µl each nostril)	Baseline to 100% (20 wk p.i.)	Range, 90 to 100% of disease course (18 to 20 wk p.i.)
Cervid and SH sham-exposed negative controls				
p.o.	WTD (2)	10% naive deer brain (1 g)		
Aerosol	WTD (3)	5% (wt/vol) naive deer brain (2 ml)		
p.o. + i.n. + i.v.	WTD (1)	10% naive brain (0.5 g) p.o. + 0.05 g i.n. + 225 ml naive deer whole blood i.v.		
p.o.	MJD (2)	10% naive brain (1 g)		
p.o. + i.n.	MJD (2)	10% naive brain (0.5 g) p.o. + 0.05 g i.n.		
Uninoculated	MJD (4)	No inoculation		
e.n.	SH (36)	10% brain homogenate from naive SH (10 µl total [5 µl to each nostril]) ^f		

^a Abbreviations: WTD, white-tailed deer; MJD, Reeves' muntjac deer; SH, Syrian hamster; i.v., intravenous; p.o., oral; i.n., intranasal; s.c., subcutaneous; e.n. extranasal.

^b Abbreviations: CWD, chronic wasting disease; HY-TME, hyper strain of transmissible mink encephalopathy; LD₅₀, 50% lethal dose.

^c Sample collected from a previous study (5).

^d Sample collected from a previous study (13).

^e Sample collected from a previous study (14).

^f See reference 19.

or more replicates for each sample came up positive as all negative controls remained at 0/8 replicates positive.

PrP^C-CCA was detected in whole blood (Fig. 1) within 0.001 to 0.0075% of the TSE disease course (15 min for deer and hamsters, respectively) in 5/5 i.v.-exposed deer (100% replicates), 17/17 mucosally exposed deer (28.24% replicates), and 3/3 hamsters (33.3% replicates). By 0.002 to 0.015% of the disease course (30 min), PrP^C-CCA was detected in 5/5 i.v.-exposed deer (97.5% replicates), 17/17 mucosally exposed deer (90.07% replicates), and 3/3 hamsters (100% replicates). Between 0.09 and 0.7% of the disease course (24 to 72 h), PrP^C-CCA was demonstrated in 2/2 or 3/3

i.v.-exposed deer (93.75 to 33.25%), 6/6 or 4/4 mucosally exposed deer (22.92 to 6.25% replicates), and 3/3 hamsters (20.88 to 8.33% replicates). Subsequent detection of PrP^C-CCA was demonstrated at 4 to 5% of the TSE disease course (5 days in hamsters and 1 month in cervids) in 3/3 deer (12.5% replicates) and 3/3 hamsters (33.3% replicates). At 50% of the disease course (cervids, 15 months p.i.; hamsters, 10 weeks p.i.), PrP^C-CCA was demonstrated in 17/17 mucosally exposed deer (85.94% replicates) and 3/3 hamsters (95.88% replicates). By ~60% of the disease course (cervids, 17 months p.i.; hamsters, 12 weeks p.i.), PrP^C-CCA was demonstrated in 90 to 100% of replicates of all TSE-infected hosts.

FIG 1 Detection of PrP^C-conversion competent amyloid (CCA) in cervids and hamsters. Blood was collected from naive and TSE-infected white-tailed deer, muntjac deer, and Syrian golden hamsters prior to (baseline or 0 min p.i.) or immediately following inoculation and throughout the course of disease until termination. All samples were run in 8 replicates via wbRT-QuIC, and replicates within each inoculation route (i.e., i.v. [IV], oral [PO], aerosol, and extranasal [EN]) were averaged together. Blood from the cervid inoculation routes was collected at selected time points: 15, 30, and 60 min p.i.; 24, 48, and 72 hpi; and 1 to 34 months p.i. (A). Blood from i.v.-exposed cervids was collected from 0 min p.i. to 72 h p.i. ($n = 2$ to 6 per time point) and from 1 to 12 months p.i. ($n = 1$ per time point). For orally exposed cervids, blood was collected from 0 min p.i. to 72 h p.i. ($n = 4$ to 17 per time point) and from 1 to 30 months p.i. ($n = 1$ to 11 per time point). For aerosol-exposed cervids, blood was collected from 1 to 12 months p.i. ($n = 6$ per time point). Blood from EN-inoculated hamsters was collected at selected time points: 15, 30, and 60 min p.i.; 48 and 72 h p.i.; 5, 7, and 10 dpi; and 2 to 20 weeks p.i. (B) For e.n.-exposed hamsters, blood was collected from 0 min p.i. to 20 weeks p.i. ($n = 3$ per time point). The early (15 min to 72 h) and middle (cervids, 1 to 17 months; hamsters, 5 days to 14 weeks) stages of disease represent average asymptomatic disease prior to neurologic symptoms. The late stage represents the average occurrence of neuroinvasion.

This is the first report of the detection of PrP^C-CCA in the blood of animals within minutes of exposure to TSE inoculum. The use of PrP^C-CCA to detect prions in tissues and bodily fluids of TSE-infected hosts has been shown to be as sensitive as a bioassay and has provided insight into a variety of prion diseases (15–18). The immediate detection of the point-source inoculum was seen following exposure to mucosal surfaces in the nose and gut; the same immediate detection of PrP^C-CCA was seen following i.v. injection. This immediate detection indicates that the mucosae of the nasal cavity and the gut provide an inefficient anatomical barrier to prion entry—i.e., exposure to the mucosal surfaces does not differ significantly, in terms of temporal systemic spread, from injection directly into blood. These data, along with reports from Kincaid et al. (19) and Jeffrey et al. (20), demonstrate that mucosal surfaces are not capable of preventing the near-immediate passage of the inoculum into underlying lamina propria and blood. The absence of an efficient mucosal barrier to prion infection has significant implications for the pathogenesis of prion diseases. In addition, there are experimental and safety concerns, including the consideration that the blood of any animal exposed to prions may immediately contain prions.

ACKNOWLEDGMENTS

We thank the animal caretakers at Colorado State University, Jeanette Hayes-Klug, Kelly Anderson, and Erin McNulty, along with those at Creighton University, for all of their work regarding animal welfare and sample collection, Amber Mayfield and Monica Brandhuber for sample setups and blinding, and Anca Selariu for editing.

Funding for this work was provided through NIH grants R01AI112956, R01NS061994, and R01AI093634.

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