HHS Public Access

Author manuscript

J Med Primatol. Author manuscript; available in PMC 2018 August 01.

Published in final edited form as:

J Med Primatol. 2017 August ; 46(4): 149–153. doi:10.1111/jmp.12295.

Emerging Diagnostic Challenges and Characteristics of Simian Betaretrovirus Infections in captive macaque colonies

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Abstract

To better understand Simian betaretrovirus (SRV) seropositivity in virus negative macaques, we transfused blood from SRV infected or suspect donors into immunosuppressed naive recipients. Our results do not support typical SRV1-5 infection as the cause, but provide evidence for several possibilities including serological artifact, new/different SRV, or an endogenous virus.

Keywords

transfusion; serology; PCR

Introduction

Simian betaretrovirus (SRV) has been a model of immunodeficiency and a target of exclusion in colony management of macaques since the 1980's [1–3]. With the implementation of improved diagnostic testing and management practices, the prevalence of naturally occurring SRV in captive colonies has declined dramatically [4, 5]. However, despite best practices, small but growing numbers of seropositive, virus-negative animals with no plausible history of exposure have been confirmed by multiple laboratories using various methods including antibody assays with viral lysate and recombinant protein targets on platforms including enzyme immunoassays, microbead arrays, immunofluorescence and Western Blot. Virus detection assays have included PCR and virus isolation in multiple cell lines [6]. While some results could perhaps be attributed to assay artifact, samples with reproducible antibody in the absence of any virus detection have been identified by all assays. These observations raise questions which our transfusion studies begin to address: Have current diagnostic methods become so exquisitely sensitive and the virus so rare that we are detecting noise? Is the host making an immune response to endogenous virus? Is this

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a new serotype? Have selection pressures changed the characteristics of the virus and/or its host?

Materials and Methods

All animals were maintained in fully AAALAC-accredited facilities in accordance with the Animal Welfare Act, Regulations, and the Guide for the Care and Use of Laboratory Animals [7–9]. All procedures involving animals in this study were approved by each institution's IACUC. As detailed in figure 1, citrated whole blood was collected from known SRV infected or SRV suspect (incomplete or full antibody reactivity patterns without PCR confirmed virus detection) pigtailed macaques and transfused into seven naive, CD8+ cell-depleted adult rhesus macaques [10]. Depletion was accomplished by the intravenous administration of monoclonal anti-CD8alpha rhesus recombinant M-T807R1 (NIH Nonhuman Primate Reagent Resource) at 50 mg/kg 48 hours prior to transfusion, to reduce CD8+ cell-mediated immune responses and increase the chances of inducing infection [11]. Animal health was monitored daily by trained animal care and veterinary staff. Blood samples for complete blood count (CBC), CD3/4/8/20 FACS analysis, SRV antibody, SRV PCR, and culture were collected at days 0, 3, 7, 10, 14, 21, 28, 35, 42 and necropsy (days 49–56). Each animal receiving known SRV positive blood was pair housed with a cage mate that was not subjected to any study manipulations. The four receiving SRV suspect blood were paired together. CBCs were performed on EDTA-anticoagulated blood using a Pentra 60C+ analyzer (ABX Diagnostics) with differentials determined manually. Lymphocyte subpopulations were analyzed by four-color flow cytometry using a FACSCalibur flow cytometer. Multiplex microbead (Charles River Laboratories, Wilmington, MA) and Western blot immunoassays were employed for antibody [13]. The microbead assays used recombinant and viral lysate antigens with biotinylated goat anti-human IgG and streptavidin peroxidase for detection on the Luminex platform. For immunoblots, SRV1 and SRV2 viral lysate were electrophoresed through a 4–12% gradient gel and transblotted onto PVDF membrane. Peroxidase conjugated goat anti-monkey IgG and 4-chloro-1-naphthol were used for detection. Real time PCR was performed using primers validated to detect SRV1-5 serotypes with a sensitivity of one to ten copies [14]. PBMCs were isolated, stimulated with SEA, and co-cultured on Raji cells in fetal calf sera supplemented RPMI media for six weeks [15, 16]. Cultures were observed twice weekly for CPE and supernatant was collected for PCR.

Results

As shown in Table 1, all animals were efficiently CD8+ cell depleted for ≥ 3 weeks. SRV viral DNA was detected directly from PBMCs and in Raji cultures beginning two weeks post transfusion in the monkeys that received either SRV1 or SRV4 virus and antibody positive pooled blood from known infected donors. Rising antibody levels were detected in those same monkeys by four weeks post transfusion. No virus or seroconversion was detected in the monkey receiving antibody positive/virus negative blood from the known SRV5 infected donors. Virus or seroconversion was also not detected in the monkeys receiving blood from SRV suspect antibody indeterminate or positive but virus negative donors. No recipients exhibited clinical or hematological signs of disease during the course

of this study. No pathology indicative of SRV infection was seen at necropsy. Furthermore, none of the newly SRV infected macaques transmitted infection to their naïve cage mates. However, subsequent transfusion from these known SRV recipients into another generation of naïve macaques did result in the expected new infections with SRV1 and 4 but not SRV5.

Discussion

Although SRV1 and 4 infection were successfully transmitted from donor pools to recipient animals, the demonstrated lack of transmission to cage mates and lack of pathology (i.e. anemia, immunosuppression, wasting) contrasts with the historical descriptions of SRV [1, 2]. Could evolutionary pressures over time be selecting out a specific population of macaques or virus strains? The inability to confirm infection by either antibody or PCR in any recipients of SRV suspect blood supports the possibilities that these non-negative antibody/PCR negative profiles do not indicate conventional SRV1-5 infection but could perhaps represent a serological assay artifact; a different, low level, difficult to detect virus; or reactivity to an endogenous virus. With the current exquisitely sensitive diagnostic methods and low virus prevalence in many established colonies, statistical principles favor the greater probability of false as compared to true positives [6, 17, 18]. Recent publications have reported additional serotypes beyond the well-established SRV1-5 [19–23]. We (R. Grant, manuscript submitted) and others have demonstrated confounding detection of host immune responses to endogenous viruses in SRV serology [24, 25]. Although both SRV5 donors were antibody positive/PCR negative on the day of transfusion, they were infected as shown by not only antibody but also PCR positivity on five of ten and one of ten dates tested during the prior two months. Without a better understanding of the mechanisms and stresses triggering PCR positivity at any time point [26], transmission risks remain [26, 27]. Studies to further address and differentiate possible explanations to unravel the mystery of SRV seropositive/virus-negative and potential infection risk to other macaques are in progress.

Acknowledgments

We thank the animal care and laboratory staff at both the California and Washington National Primate Research Centers for providing excellent technical support for this study. This work was supported by NIH grants CNPRC P51 OD011107 and WANPRC P51OD010425.

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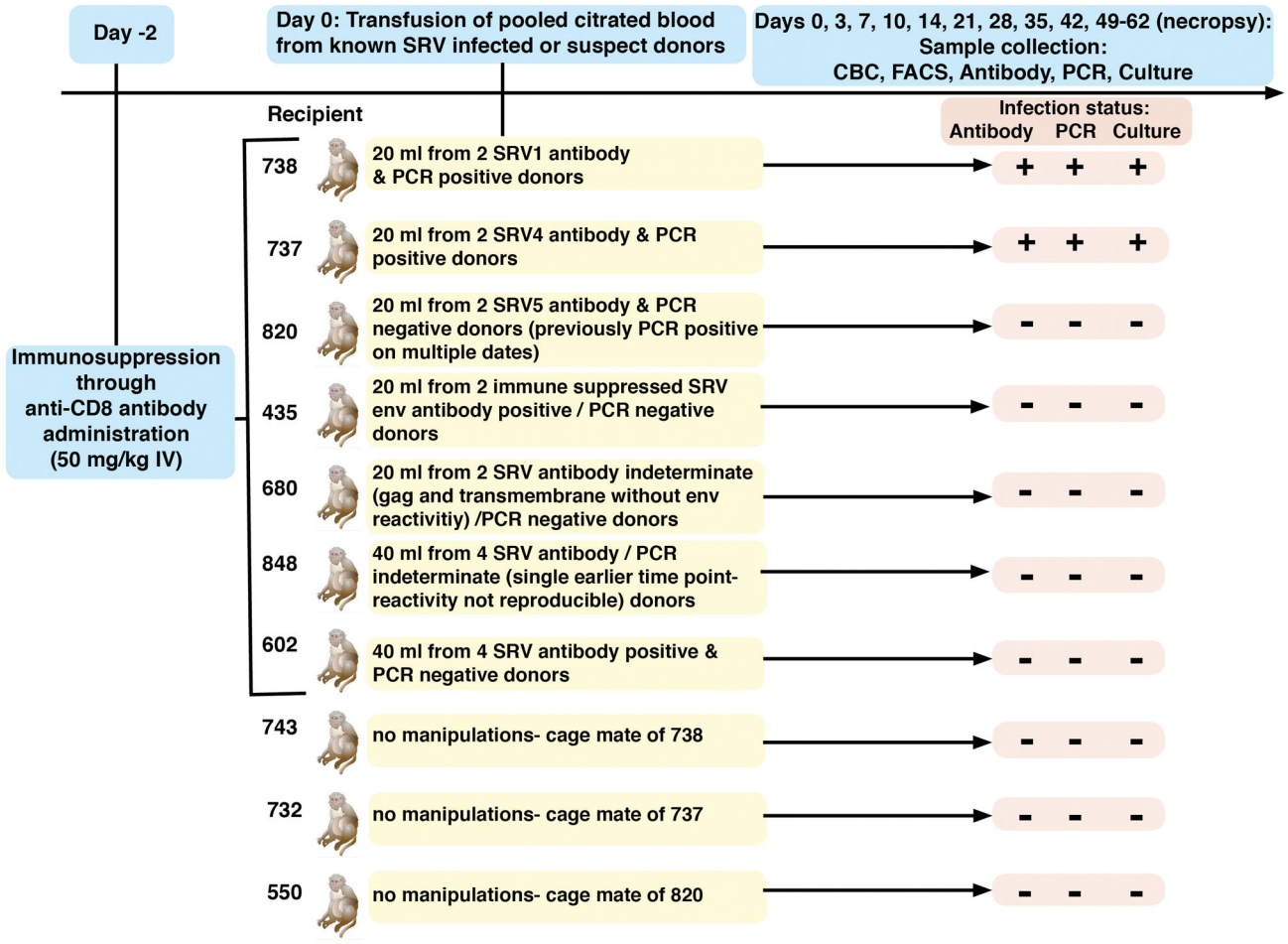

Figure 1.

Table 1

	Day −2	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Necropsy
MMU 738: received known SRV1 (antibody & PCR positive) transfusion)									
Antibody (WB bands)		neg		POS	POS	POS	POS	POS	POS
PCR (Ct <55 = POS)		neg	neg	POS (37)	POS (31.6)	POS (26.5)	POS (27.5)	POS (25.5)	POS (24.8)
Raji Cell Culture		neg	neg	POS	POS	POS	POS	POS	POS
Health Report		normal	normal	normal	normal	normal	normal	normal	normal
CBC: RBC#/Hct%/WBC#/Lymph %	4.91/33.3/8.7/15	5.23/36.0/6.5/17	5.32/36.3/7.6/20	4.87/33.3/6.5/21	5.34/36.5/9.6/16	5.15/34.2/5.3/27	4.67/30.9/7.3/53	5.05/33.1/9.0/15	
FACS: 3+4+/3+8+/3–20+/3–8+(NK)	366/322/447/154	510/8/499/9	839/4/586/3	682/4/560/1	880/32/486/14	525/161/525/111	885/722/828/1471	342/194/276/575	
MMU 737: received known SRV4 (antibody & PCR positive) transfusion									
Antibody (WB confirmed)		neg		none	POS	POS	POS	POS	POS
PCR (Ct <55 = POS)		neg	neg	Ind (48)	POS (38.3)	POS (28.8)	POS (30.1)	POS (30.0)	POS (30.6)
Raji Cell Culture		neg	neg	POS	POS	POS	POS	POS	POS
Health Report		normal	normal	normal	normal	normal	normal	normal	normal
CBC: RBC#/Hct%/WBC#/Lymph %	4.63/34.9/3.6/46	5.22/39.8/5.3/19	5.12/38.5/4.7/29	4.46/33.3/3.2/41	4.85/36.3/2.8/44	5.12/37.9/3.2/53	4.73/34.7/4.3/44	4.53/32.9/3.8/50	
FACS: 3+4+/3+8+/3–20+/3–8+(NK)	582/417/346/291	521/4/371/2	941/7/315/1	795/2/351/1	819/2/280/1	1111/2/426/1	1075/8/560/2	828/204/522/369	
MMU 820: received known SRV5 (antibody positive/PCR negative) transfusion									
Antibody (WB bands)		neg		neg	neg	neg	neg	neg	neg
PCR (Ct <55 = POS)		neg	neg	neg	neg	neg	neg	neg	neg
Raji Cell Culture		neg	neg	neg	neg	neg	neg	neg	neg
Health Report		normal	normal	normal	normal	normal	normal	normal	normal
CBC: RBC#/Hct%/WBC#/Lymph %	5.03/35.2/4.09/35	5.46/38.5/8.9/14	5.25/36.7/6.0/24	5.08/35.5/3.7/44	5.53/38.6/4.6/35	5.25/36.7/3.4/38	5.30/37.0/4.0/31	5.32/37.2/4.8/58	4.90/34.0/4.2/57

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	Day −2	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Necropsy
FACS: 3+4+/3+8+/3–20+/3–8+(NK)	88/416/325/169	726/9/388/2	1075/5/260/1	1205/9/268/0	1252/5/208/0	880/6/259/2	819/3/262/1	1793/12/604/5	
MMU 435: received Suspect SRV (env Ab positive/PCR negative) transfusio									
Antibody (WB confirmed)		neg		neg	neg	neg	neg	neg	neg
PCR (Ct <55 = POS)		neg	neg	neg	neg	neg	neg	neg	neg
Raji Cell Culture		neg	neg	neg	neg	neg	neg	neg	neg
Health Report		normal	normal	normal	normal	normal	normal	normal	normal
CBC: RBC#/Hct%/WBC#/Lymph %	5.35/39.1/4.7/38	5.87/42.8/5.1/31	5.21/37.7/4.0/52	4.78/34.2/4.7/57	5.17/37.1/5.6/59	5.43/38.6/5.8/40	5.20/37.1/4.5/62	5.31/38.1/5.8/43	
FACS: 3+4+/3+8+/3–20+/3–8+(NK)	838/2389/392/213	986/9/465/3	1403/7/534/2	1705/8/639/0	1877/5/1143/2	1123/14/854/8	1556/13/934/3	1395/202/803/77	
MMU 680: received Suspect (SRV gag and transmembrane Ab positive/PCR negative) transfusion									
Antibody (WB bands)		neg		neg	neg	neg	neg	neg	neg
PCR (Ct <55 = POS)		neg	neg	neg	neg	neg	neg	neg	neg
Raji Cell Culture		neg	neg	neg	neg	neg	neg	neg	neg
Health Report		normal	normal	normal	normal	normal	normal	normal	normal
CBC: RBC#/Hct%/WBC#/Lymph %	4.70/34.9/5.9/19	5.36/40.0/5.9/17	4.93/36.5/6.4/28	4.53/33.5/5.8/33	4.81/35.6/5.2/43	5.02/37.2/7.4/25	4.83/35.5/6.4/48	4.59/34.1/7.8/38	4.56/33.5/6.4/36
FACS: 3+4+/3+8+/3–20+/3–8+(NK)	502/164/296/134	524/3/361/2	965/7/706/3	1054/6/604/0	1243/2/657/2	889/5/546/3	1591/365/500/462	1259/293/644/662	
MMU 848: received (Suspect SRV Ab positive/PCR*) transfusion *single unconfirmed, non-reproducible PCR signal at an earlier date									
Antibody (WB bands)		neg		neg	neg	neg	neg	neg	neg
PCR (Ct <55 = POS)		neg	neg	neg	neg	neg	neg	neg	neg
Raji Cell Culture		neg	neg	neg	neg	neg	neg	neg	neg
Health Report		normal	normal	normal	normal	normal	normal	normal	normal

	Day −2	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Necropsy
CBC: RBC#/Hct%/WBC#/Lymph %	5.37/39.4/9.7/46	5.24/38.1/4.4/50	4.82/35.0/7.9/28	4.67/34.0/5.8/48	5.25/38.2/8.6/43	5.60/40.6/8.2/43	5.19/37.2/6.2/46	4.80/34.9/7.4/34	4.58/33.0/6.3/66
FACS: 3+4+/3+8+/3–20+/3–8+(NK)	1414/616/1759/602	861/10/1161/3	1054/6/1024/6	1160/6/1353/3	1737/9/1389/2	1610/8/1356/10	1390/155/975/212	1191/162/971/157	
MMU 602: received Suspect (SRV Ab positive/PCR negative) transfusion									
Antibody (WB bands)		neg		neg	neg	neg	neg	neg	neg
PCR (Ct <55 = POS)		neg	neg	neg	neg	neg	neg	neg	neg
Raji Cell Culture		neg	neg	neg	neg	neg	neg	neg	neg
Health Report		normal	normal	normal	normal	normal	normal	normal	normal
CBC: RBC#/Hct%/WBC#/Lymph %	5.19/38.4/5.8/49	5.31/39.35/7.4/30	5.08/37.3/8.6/23	4.61/33.7/5.4/51	5.16/37.4/7.1/41	5.34/38.9/8.9/26	5.09/37.2/8.6/25	4.82/35.5/7.2/33	
FACS: 3+4+/3+8+/3–20+/3–8+(NK)	807/491/1339/140	609/11/1445/12	700/7/1159/3	846/7/1631/2	696/5/2018/5	535/7/1595/7	496/3/1483/6	676/3/1531/5	