Pathogenesis of pulmonary arteriopathy in lentivirus-infected pigtailed macaques (*M. nemestrina*)

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BACKGROUND

A syndrome of proliferative occlusive pulmonary arteriopathy involving thrombi in the pulmonary trunk and larger pulmonary arteries in conjunction with peripheral thrombocytopenia occurs with varying frequency in lentivirus-infected macaques. It can result in the sudden demise of an animal with no previous clinical signs. Such losses present an animal welfare concern, and result in the loss of valuable research data. In addition, pulmonary hypertension and thromboembolic disease are potentially-fatal consequences of HIV infection in humans. As survival rates of HIV-infected patients improve, the incidence of these complications is increasing. Thrombocytopenia is also a consequence of HIV infection in humans. Although there are retrospective reports concerning these diseases in humans, it is not possible to perform a comprehensive, prospective experiment in humans.

PURPOSE AND HYPOTHESIS

Based on this preliminary data and review of the literature, we hypothesize a multifactorial etiology for the development of pulmonary arteriopathy and thrombosis in lentiviral-infected macaques: Lentivirus infection induces a chronic inflammatory state associated with microbial translocation, and the inflammatory mediators promote a hypercoagulable state. We further hypothesize that the direct and indirect effects of lentivirus on the vascular endothelium of the medium and large pulmonary arteries induces chronic changes which not only result in slow development of pulmonary arteriopathy characterized by intimal and medial proliferation with minimal inflammatory cell infiltration, but also increase platelet activation resulting in platelet aggregation and consumption of platelets. This chronic arteriopathy and thrombosis increases right ventricular afterload, resulting in dilatation of the right side of the heart and increased pulmonary arterial pressure.

MATERIALS AND METHODS

Data were collected from two cohorts of experimental animals:

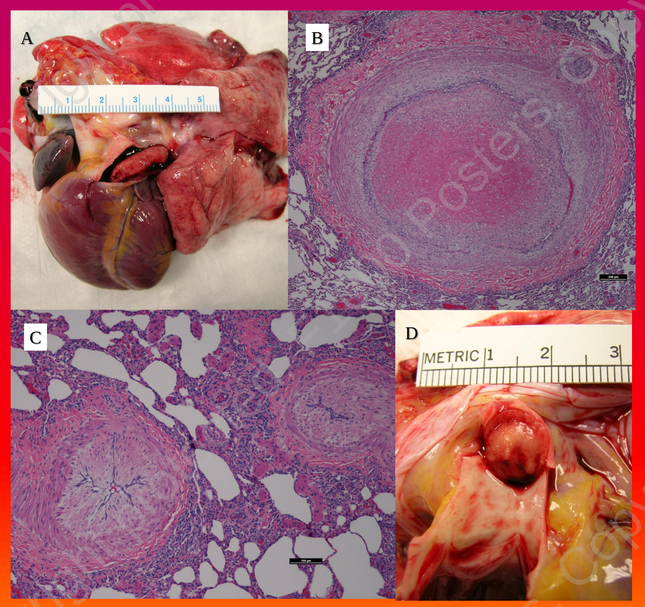
1) Retrospective analysis of hematological and pathological data
   - Vaccine trial
   - 42 *Macaca nemestrina*
   - Inoculated intravenously with SIV_{mne}B3718
   - Followed until clinical deterioration compelled euthanasia
   - Peripheral blood samples were collected in appropriate anti-coagulants and assayed for CBC with T cell subunits, serum chemistry, and viral load throughout the study.
   - All animals underwent necropsy and histologic examination at termination.
2) Echocardiography and coagulation-related hematologic parameters
   - Vaccine trial: protein, DNA, and/or vaccinia vector containing viral DNA; gag, pol, and env genes included
   - 23 *Macaca nemestrina*
   - Inoculated intrarectally with SHIV SF162P4 (env from HIV-1-SF162; gag, pol from SIV_{mac239})
   - Animals were assessed three times at monthly intervals 2-3 years post inoculation and euthanized
   - Peripheral blood samples were collected in appropriate anti-coagulants and assayed for CBC with T cell subunits, serum chemistry, viral load, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, antithrombin (AT), protein C activity, protein S activity, antiphospholipid antibodies, D-dimer, von Willebrand Factor antigen (vWF), and thromboelastography (TEG).
   - A Phillips HD-11XE ultrasound machine with an S8-3 MHz transthoracic probe and an S7-2 MHz transesophageal probe were used to perform 2-D and Doppler echocardiography.
   - 21 animals – no evidence of pulmonary arteriopathy
   - 2 animals – euthanized early due to pulmonary arteriopathy – partial data sets collected

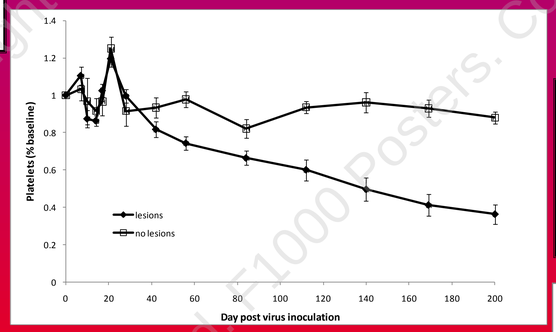
RESULTS

Number of *M. nemestrina* infected with SIV_{mne} with or without pulmonary arteriopathy and thrombocytopenia. Thrombus/arteriopathy is significantly associated with thrombocytopenia (P < 0.001 by Fisher's exact test)

	No pulmonary vascular lesions	Thrombus/ arteriopathy
Platelet decrease > 50%	1	32
No platelet decrease	8	1

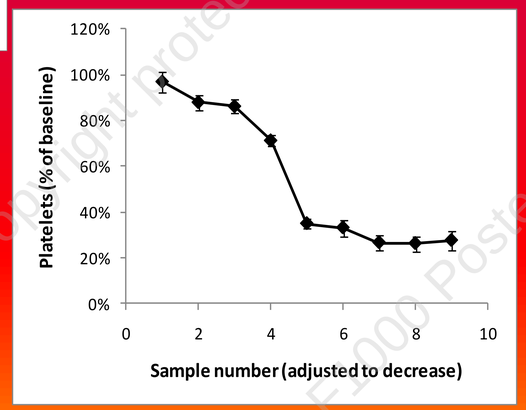
Pulmonary arteriopathy in lentivirus-infected *M. nemestrina*. A+D) Thrombus extending from the right ventricle into the pulmonary trunk. B) Thrombus occluding a pulmonary artery. C). Medial and intimal proliferation of pulmonary artery with minimal inflammatory response.





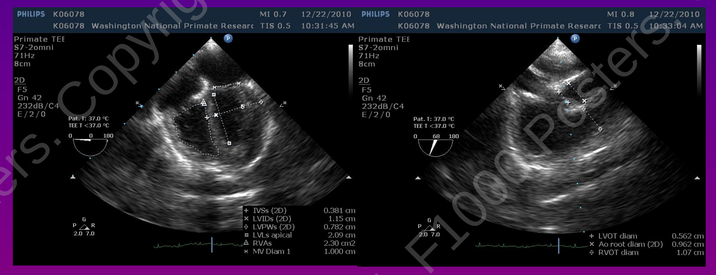
Change in platelet counts in *M. nemestrina* following inoculation with SIV_{mne} normalized to the first sample with a platelet count less than 50% of baseline. Each animal exhibits a sudden decrease in platelets rather than a slow decline, as can be seen when data are synchronized to the first blood sample with a platelet count lower than 50% of baseline. For this figure, platelet counts were converted to percentage of the day 0 platelet count, and the first sample with a value lower than 50% was identified. The preceding four samples and the following four samples for each animal were selected, and means were calculated based on temporal relationship to the index sample. (Mean ± SEM, n = 32)

Changes in platelet count over time as a percentage of baseline in *M. nemestrina* following inoculation with SIV_{mne}. Data (mean ± SEM) are shown for animals with thrombi and/or pulmonary arteriopathy identified at necropsy as well as a >50% decrease in platelets ("lesions", n = 32) and for animals without lesions or platelet decrease ("no lesions", n = 7).



RESULTS

Echocardiography: Right ventricular (RVDd, RVAd, RVAs) dilatation and thickening of the right ventricular free wall (RVFWd) in animals with pulmonary arteriopathy.



	No clinical signs (N = 21)	Pulmonary arteriopathy (N = 2)
RVFWd (cm)	0.33 (0.05)	0.40 (0.06)[a]
RVDd (cm)	1.25 (0.19)	1.65 (0.28)[a]
LVDd (cm)	1.11 (0.24)	1.38 (0.20)
RVD/LVD	0.75 (0.17)	0.92 (0.13)
RA diam. (cm)	1.78 (0.29)	2.44[b]
RVAd (cm^2)	2.54 (0.63)	4.33 (0.80)[a]
RVAs (cm^2)	1.59 (0.44)	2.61 (0.21)[a]
FAC RV (%)	36 (16)	39 (6)

[a]Significantly different from normal cohort by t-test
[b]Only one animal measured

Table 3. Coagulation parameters from *M. nemestrina* inoculated with SHIV-SF162.

	Platelet ($x10^3$)	PT (sec)	aPTT (sec)	D-dimer (ug/ml FEU)	AT (%)	Protein C (%)	Protein S (%)	vWF (%)
Uninfected	472 (113)	12.6 (0.9)	25 (3)	0.70 (0.30)	108 (13)	219 (24)	121 (16)	269 (60)
Infected, no clinical signs	394 (95)	13.5 (0.9)	25 (3)	0.40 (0.26)	107 (11)	203 (34)	138 (21)	305 (63)
Pulmonary arteriopathy	93 (78)	16.0 (2.9)	44 (13)	2.47[b]	76[b]	100[b]	93[b]	515[b]

CONCLUSIONS

We have found that animals that develop pulmonary arteriopathy demonstrate a marked decrease in platelets prior to the development of clinical signs. This decrease in platelet count is associated with the development of pulmonary arteriopathy/thrombosis with a positive predictive value of 97%. We have also found abnormalities in coagulation parameters and echocardiographic measurements in animals in the terminal stages of arteriopathy, including increased PT, PTT, d-dimer, and VWF, and dilatation of the right ventricle.

BIBLIOGRAPHY

1. Chalifoux, L.V., et al., *Arteriopathy in macaques infected with simian immunodeficiency virus.* Lab Invest, 1992. **67**(3): p. 338-49.
2. George, M.P., et al., *Pulmonary vascular lesions are common in SIV- and SHIV-env-infected macaques.* AIDS Res Hum Retroviruses, 2011. **27**(2): p. 103-11.
3. Marecki, J.C., et al., *HIV-1 Nef is associated with complex pulmonary vascular lesions in SHIV-nef-infected macaques.* Am J Respir Crit Care Med, 2006. **174**(4): p. 437-45.
4. Metcalf Pate, K.A., C.N. Morrell, and J.L. Mankowski, *Characterization of platelet decline during acute simian immunodeficiency virus infection in the pig-tailed macaque (Macaca nemestrina).* J Am Assoc Lab Anim Sci, 2009. **48**(5): p. 625.
5. Sehgal, P.B., et al., *Golgi dysfunction is a common feature in idiopathic human pulmonary hypertension and vascular lesions in SHIV-nef-infected macaques.* Am J Physiol Lung Cell Mol Physiol, 2009. **297**(4): p. L729-37.
6. Shannon, R.P., et al., *Dilated cardiomyopathy associated with simian AIDS in nonhuman primates.* Circulation, 2000. **101**(2): p. 185-93.
7. Wachtman, L.M., et al., *Platelet decline: an early predictive hematologic marker of simian immunodeficiency virus central nervous system disease.* J Neurovirol, 2006. **12**(1): p. 25-33.
8. Yanai, T., et al., *Systemic arteriopathy in SIV-infected rhesus macaques (Macaca mulatta).* J Med Primatol, 2006. **35**(2): p. 106-12.
9. Hukkanen, R.R., et al., *Comparative incidence of proliferative-occlusive vasculopathy and thromboembolic myocarditis in pig-tailed macaques inoculated with different lentiviral strans: HIV-2 287, SHIV SF162P3, SHIV 89.6P or SIVmne.* J. Med. Primatol., 2007. **36**(4-5): p. 317.
10. Hukkanen, R.R., et al., *Proliferative-occlusive arteriopathy in pig-tailed macaques (Macaca nemestrina) infected with SIVmne.* J. Med. Primatol., 2007. **36**(4-5): p. 317.
11. Alcantara, S., et al., *Thrombocytopenia is strongly associated with simian AIDS in pigtail macaques.* J. Acquir. Immune Defic. Syndr., 2009. **51** (4): p. 374-9.