Platelet-endothelial interactions in SIV-associated CNS disease

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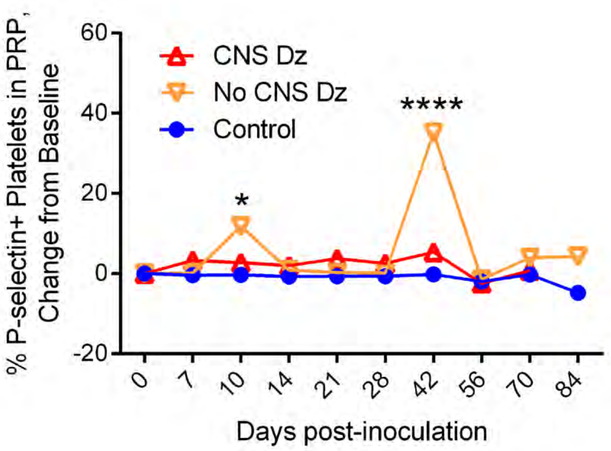
Introduction:

Platelet decline is predictive for the later development of HIV-associated CNS disease

- Combined antiretroviral therapy (cART) prevents the replication of HIV, allowing HIV infected individuals to live for many years. However, incidence of HIV-associated neurocognitive deficits remains high.[1]
- A decline in platelet numbers is one of the first hematologic signs of HIV infection, and decline in asymptomatic infection is predictive for development of HIV-associated CNS disease.[2-4]
- Decreased production and increased destruction contribute to platelet decline (HIV-associated thrombocytopenia or HAT) during asymptomatic lentiviral infection.[5]
- In the SIV infected macaque model of HIV-associated CNS disease, the formation of activated platelet – CD16+ monocyte aggregates drives platelet decline during acute infection.[4]
- Platelet binding alters the phenotype of other cells, including monocytes and endothelium.[6]

Hypothesis:
Activated platelet-endothelial associations contribute to SIV-associated CNS disease

Platelets are less activated during acute and asymptomatic infection in macaques that later develop SIV-associated CNS disease



Pigtailed macaques were dual-inoculated on day 0 with the neurotropic viral clone SIV/17E-Fr and the immunosuppressive viral swarm SIV/B670. Uninfected control macaques were mock-inoculated with 0.9% saline.

Blood was harvested from the femoral vein at each timepoint directly into a vacutainer containing sodium citrate at a 1:10 dilution using a 21 gauge 1.5 inch needle. After discarding the first 2.0 mL of blood to exclude , platelet rich plasma was harvested by centrifugation, and stained with anti-p-selectin antibody for flow cytometry analysis within one hour of collection.

To determine CNS disease status, macaque brains harvested during necropsy at terminal infection were examined by two veterinary pathologists. Slides were blinded and scored for the presence of perivascular mononuclear infiltrates characteristic of SIV-associated CNS disease.

Two way ANOVA, N = 5 with CNS disease, N = 5 without CNS disease, N = 5 uninfected controls, *P = 0.048, ****P < 0.0001.

SIV infected macaques are more likely to have platelet-lined vasculature in the brain than uninfected macaques, starting during acute infection

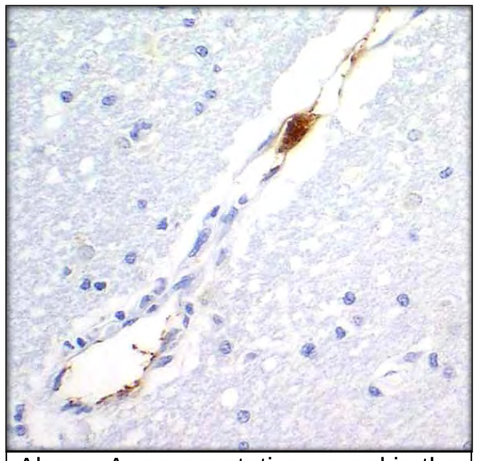
Acute Infection	Platelet-Lined Vessels	No Platelet-Lined Vessels
SIV Infected, d10 Post-Inoculation	6	0
Uninfected Controls	1	3

Fisher's Exact RR = 4.0, *P* = 0.03

Terminal Infection	Platelet-Lined Vessels	No Platelet-Lined Vessels
SIV Infected, d84 Post-Inoculation	9	1
Uninfected Controls	1	3

Fisher's Exact RR = 3.6, *P* = 0.04

CD42b+ platelets were visualized in the frontal cortex of brains from SIV infected macaques during acute (10 days post-inoculation) and terminal (84 days) infection and in uninfected controls using immunohistochemistry. Slides were scored for the presence or absence of platelet-endothelial associations by a blinded observer.



Above: A representative vessel in the frontal cortex of an SIV-infected macaque, lined by platelets (CD42b+, brown) and a platelet microthrombus.

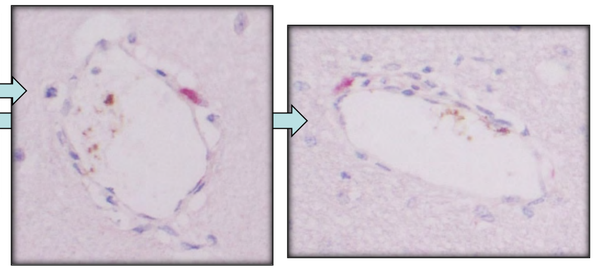
To determine whether the presence of platelets bound to endothelium is associated with the presence of the perivascular macrophage infiltrate characteristic of SIV-associated CNS disease, frontal cortex from seven terminally infected (day 84 post-inoculation) macaques with CNS disease were co-stained for CD42b+ platelets (brown) and markers for recently infiltrated non-resident classically activated M1-like macrophages (Mac387+), non-resident alternatively activated M2-like macrophages (CD163+), or macrophages in general (CD68+) (red). 500 fields per animal were randomly chosen using a motorized stage and Stereo Investigator software. Individual vessels were examined at 400X and scored for the presence of platelets bound to endothelium and / or perivascular macrophages by a blinded observer.

To determine whether these macrophages were SIV infected, frontal cortex was co-stained for CD42b+ platelets (brown) and SIV protein KK41 (red). 500 fields randomly chosen and individual vessels scored by blinded observer as above.

Platelet-endothelial associations correspond with accumulation of perivascular non-resident macrophages in the brain

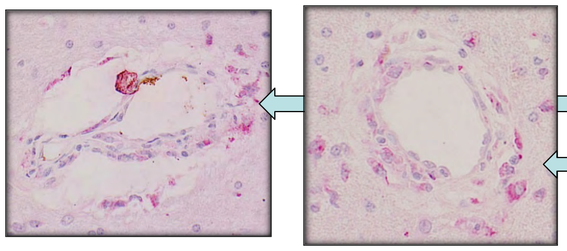
Mac387+ M1-Like Macrophages	Perivascular Mac387+ Cells	No Perivascular Mac387+ Cells
Platelets bound to endothelium	17	158
No platelets bound to endothelium	33	1114

Fisher's Exact RR = 3.4, *P* = 0.0001



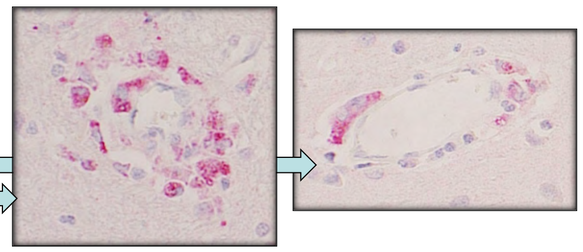
CD163+ M2-Like Macrophages	Perivascular CD163+ Cells	No Perivascular CD163+ Cells
Platelets bound to endothelium	80	103
No platelets bound to endothelium	357	819

Fisher's Exact RR = 1.4, *P* = 0.0005

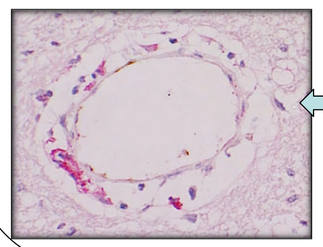


CD68+ General Macrophages	Perivascular CD68+ Cells	No Perivascular CD68+ Cells
Platelets bound to endothelium	56	58
No platelets bound to endothelium	392	541

Fisher's Exact RR = 1.2, *P* = 0.16



Platelet-endothelial associations correspond with the presence of perivascular SIV-infected macrophages

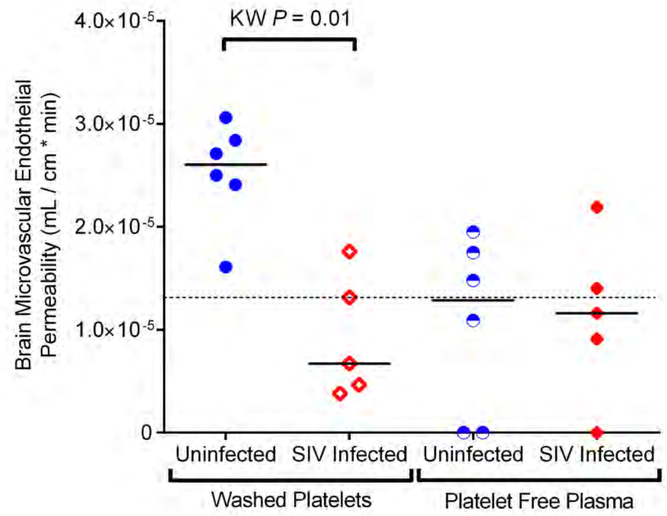


KK41+ SIV Infected Cells	Perivascular SIV Infected Cells	No Perivascular SIV Infected Cells
Platelets bound to endothelium	42	101
No platelets bound to endothelium	186	776

Fisher's Exact RR = 1.5, *P* = 0.007

Key for IHC images:
Brown: CD42b+ platelets
Red: Macrophages, marker as indicated

Washed platelets from SIV infected macaques do not increase the permeability of brain microvascular endothelium in culture



Dashed line (- - - - - - -) indicates baseline permeability after incubation with platelet-free media control.

Whole blood was harvested from 5 viremic SIV infected pigtailed macaques and 6 uninfected healthy age and sex matched controls. Platelets were washed twice and resuspended in Tyrode's albumin buffer with apyrase prior to co-culture with confluent monolayers of macaque brain endothelial cells (23B-As) in transwells; platelet free plasma harvested from the same animals and platelet free media were used as negative controls. The passage of FITC-inulin was measured over 6 hours and normalized to a cell-free transwell to calculate brain microvascular endothelial permeability.

Conclusion:

Activated platelet-endothelial associations may be protective in SIV-associated CNS disease

- Given that platelet activation predicts protection from later development of CNS disease and does not increase the permeability of brain microvascular endothelium in culture, platelet binding to vessels with perivascular macrophage cuffs may represent a protective response.
- The platelet decline during asymptomatic infection that has been associated with the later development of CNS disease may represent the loss of a protective mechanism and contribute to the development of CNS disease.
- Treatment of destruction and decreased production contributing to "sub-clinical" platelet decline during asymptomatic infection may therefore be warranted.
- Additional examination of CNS outcomes in SIV-infected macaques that are platelet depleted, platelet transfused or treated with production stimulating and destruction mitigating therapies is warranted to determine whether adjunct therapy for platelet decline may improve CNS outcomes.

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