Platelet-endothelial interactions in SIV-associated CNS disease

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Introduction

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

- SIV-infected pigtailed macaques are a model for HIV-associated CNS disease[1]
- The brain is a sanctuary organ for latent virus, presenting a barrier to cure of HIV and SIV[1]
- SIV viral entry into the brain occurs during early infection via monocytes, which form perivascular infiltrates of macrophages[1]
- HIV vDNA in the brain of virally suppressed individuals has been demonstrated exclusively within macrophages/microglia[10]
- Decreased platelet count (thrombocytopenia) occurs early in HIV infection[2] and in the SIV-infected macaque model[3] of HIV, and is persistent throughout infection
- Macaques with a greater magnitude of thrombocytopenia in asymptomatic infection are more likely to develop perivascular infiltrates than those with less severe thrombocytopenia[3]
- In asymptomatic infection, decline is linked to decreased production and increased immune-mediated destruction along with platelet-monocyte aggregate (PMA) formation[4]
- Platelets can be sequestered in associations with other cells, further contributing to platelet decline
- Platelet-endothelial associations (PEAs) have the potential to impact the integrity of endothelial barriers – they increase permeability in Dengue viral infection[5], transplant rejection[6], and rheumatoid arthritis[7]; they are shown to increase permeability of the blood-brain-barrier in malaria infection[8]
- Soluble CD40L has been shown to increase endothelial permeability in the brain in the EcoHIV model in mice[9]

Hypothesis

Platelet activation and PEA formation contributes to HIV-associated CNS disease by increasing the permeability of the blood-brain barrier.

Results

Platelets are less activated in macaques that later develop SIV-associated CNS disease during acute (10 day) and asymptomatic (42 day) infection. Activation is essential for platelets to form associations with endothelial cells.

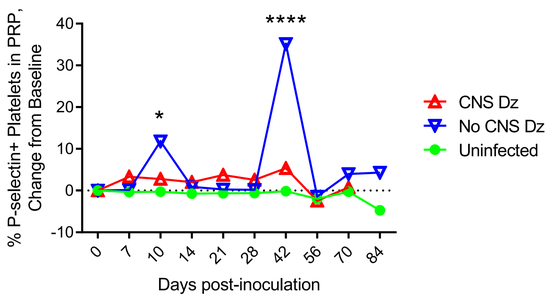


Figure 1 – Platelet activation over time following SIV infection

Percentage of platelets expressing P-selectin was measured via flow cytometry prospectively over the course of infection. CNS disease status was determined following necropsy based on the presence of perivascular mononuclear infiltrates characteristic of SIV-associated CNS disease by two blinded pathologists. n = 5 animals per group above.

Results

PEA formation with platelets from SIV-infected macaques protect against increased endothelial cell permeability. Platelets from uninfected control macaques, however, caused an increase in permeability of the endothelial cell layer. The effect of platelet-free plasma from the same animals was measured as a control, and all conditions compared to a media control (dashed line).

When direct interactions between platelets and endothelial cells were blocked, the protective effect was lost. Platelets from SIV-infected macaques caused an increase in endothelial permeability in this system that was higher than with the addition of ethanol.

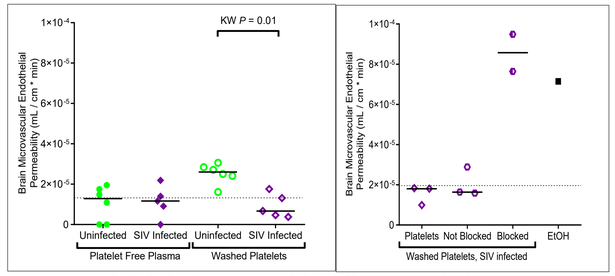


Figure 2 – Endothelial culture permeability following platelet binding in a transwell system

Left: 23B-A macaque microvascular brain endothelial cells were grown to confluence on the top layer of a transwell system, and washed platelets or platelet-free plasma were added. Platelet binding to endothelial cells was confirmed with immunofluorescence imaging for CD42b. Permeability was measured by the passage of FITC-inulin from the top chamber through the endothelial cells into the lower chamber of the transwell system.

Right: A second layer was added to the top of the transwell system with smaller pores that allowed diffusion of media but blocked platelets from accessing endothelial cells. Permeability was measured as described above. These results were compared to a "not blocked" condition consisting of a second layer with pores large enough to allow the passage of platelets. Ethanol was utilized as a positive control for endothelial permeability..

PEAs are more likely to be observed in the brain of SIV-infected than in uninfected macaques. PEA formation starts in acute and persists into terminal infection.

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

Fisher's Exact RR = 3.6, P = 0.04

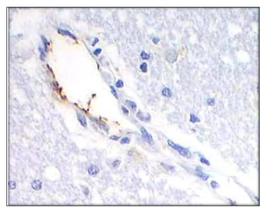
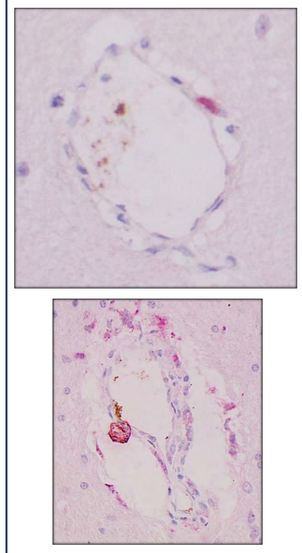


Figure 3 – PEAs in a representative vessel in the frontal cortex of an SIV-infected macaque

Sections of frontal cortex from SIV-infected macaques from acute and terminal infection as well as uninfected control animals were stained with anti-CD42b antibody.

PEAs are more likely to be found at vessels with non-resident macrophages in SIV-infected macaque brains. M1-like macrophages were defined as Mac387 positive, and M2-like macrophages were defined as CD163 positive. Macrophages as a whole were defined as CD68 positive.



Macrophages as a Whole	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

Non-Resident 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

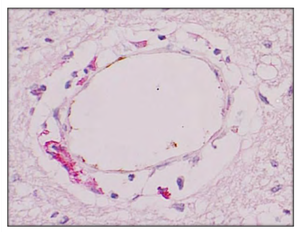
Non-Resident M2-Like Macrophages	Perivascular CD163+ Cells	No Perivascular CD163+ Cells
Platelets bound to endothelium	80	103
No platelets bound to endothelium	357	819

Fisher's Exact RR = 1.4, P = 0.0005

Figure 5 – Representative image of PEAs in a vessel with a non-resident Mac387+ macrophage (top) and CD163+ macrophage (bottom) in the frontal cortex of an SIV-infected macaque

Sections of frontal cortex were stained with anti-CD42b antibody and an antibody specific for macrophages (either CD68, Mac387, or CD163).

PEAs are more likely to be found at vessels with SIV-infected perivascular macrophages. SIV-infected cells were identified with an antibody raised against KK41, which recognizes Gp41, an envelope protein on the SIV virus.



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

Figure 6 – Representative image of PEAs in a vessel with an SIV-infected macrophage in the frontal cortex of an SIV-infected macaque

Sections of frontal cortex were stained with anti-CD42b antibody and anti-KK41 antibody.

PEAs are found in brains from people living with HIV (PLWH). Unstained sections of frontal cortex or precentral gyrus were obtained from the National NeuroAIDS Tissue Consortium (NNTC) from five groups of individuals, outlined below.

Group	Characteristics
1	HIV positive, HIV encephalitis (HIVE, either in presence or absence of any other pathologies), any neurocognitive status, no current ARV medication within 6 months of death, detectable CSF VL (>50 copies/mL) within 6 months of death.
2	HIV positive, no HIVE, no other pathologies other than 'other non-infectious pathologies' and 'minimal non-diagnostic abnormalities', consistent normal neurocognitive status, no current ARV medication within 6 months of death, undetectable CSF VL (<=50 copies/mL) within 6 months of death.
3	Same as Group 1 but consistent ARV history (at least one current medication at each reported visit PLUS last current record within 6 months of death).
4	Same as Group 3 but consistent ARV history (at least one current medication at each reported visit PLUS last current record within 6 months of death).
5	HIV negative, no other pathologies other than 'other non-infectious pathologies' and 'minimal non-diagnostic abnormalities', consistent normal neurocognitive status or no data available.

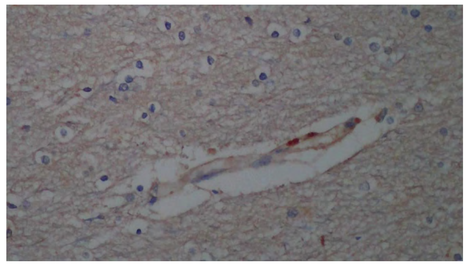


Figure 5 – Representative image of PEAs in a vessel in the frontal cortex of a HIV positive individual.

Sections of frontal cortex were obtained from the NNTC and stained with anti-CD41 antibody.

PEAs are seen in PLWH regardless of CNS disease status. Preliminary data (below) revealed PEAs in all groups, and showed that antiretroviral medication may modulate their numbers in the frontal cortex.

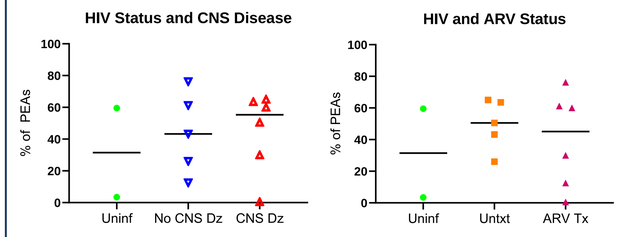
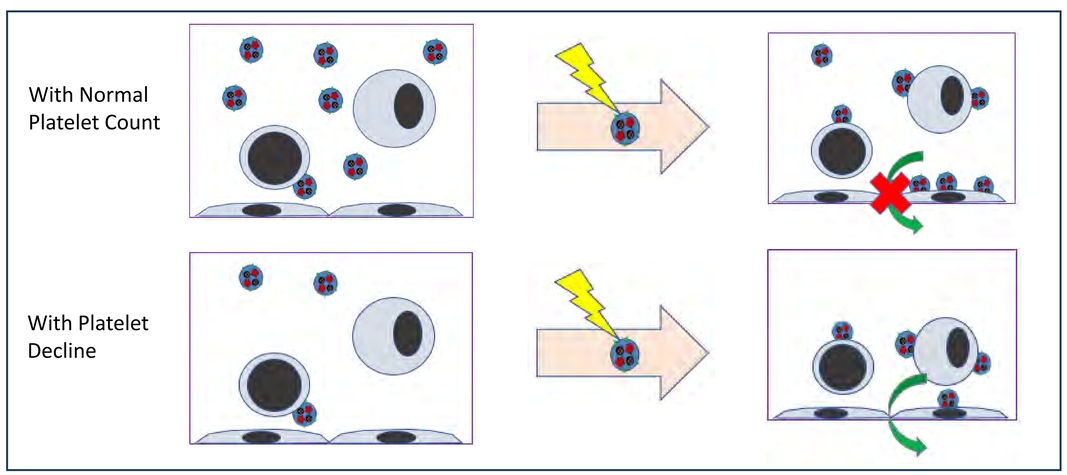


Figure 6 – Percentage of vessels with PEAs in human frontal cortex.

Slides were blinded and one individual quantified the number of blood vessels in the gray matter (up to 200 vessels) that had or did not have PEAs. Those data were unblinded and then associated with whether the individual had HIVE or was on consistent ARV treatment. The blinded slides will be quantified by three other trained individuals for full analysis.

Conclusion

Associations of activated platelets with endothelial cells in PEAs may be protective against entry of SIV-infected monocytes into the brain. Platelets were seen in the frontal cortex of PLWH with and without CNS disease, and their numbers may be modulated by ARV. Platelet decline during asymptomatic infection may lead to a loss of protection, and contribute to the development of CNS disease and viral reservoirs in the brain.



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