Adventures at the intersection of HIV, neuroinflammation and drugs of abuse: new understanding and novel therapeutics

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Despite immune recovery in individuals on antiretroviral therapy, the frequency of HIV associated cognitive disorders (HAND) remains high for reasons that are not well understood.

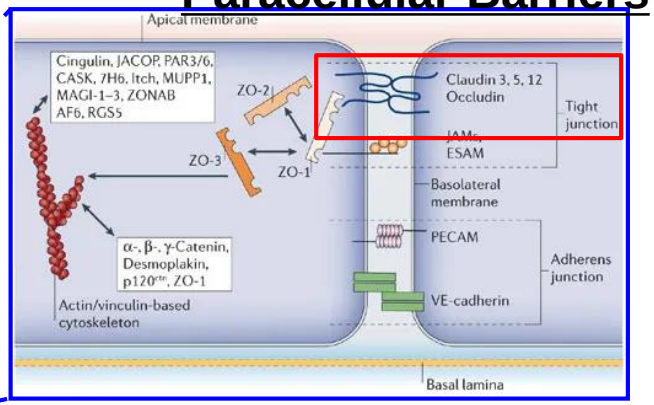
HIV-associated neurodegeneration is driven by chronic inflammatory responses in the brain secondary to:

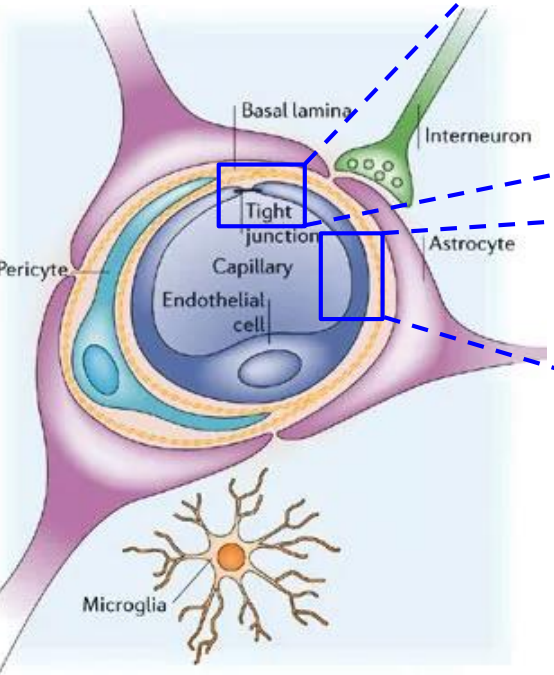
- a low level of HIV replication in CNS reservoir cells (macrophages, microglia) and
- injury to the blood brain barrier (BBB) mediated by pro-inflammatory factors in blood and migration of leukocytes across the BBB.
- BBB injury and intrathecal immune activation identified early post-infection with elevated CSF neopterin, monocyte chemotactic protein/CCL2, and interferon γ-induced protein 10/CXCL-10 levels.

Treatment approaches targeting inflammation, tightening BBB and HIV replication should be beneficial for amelioration of HAND.

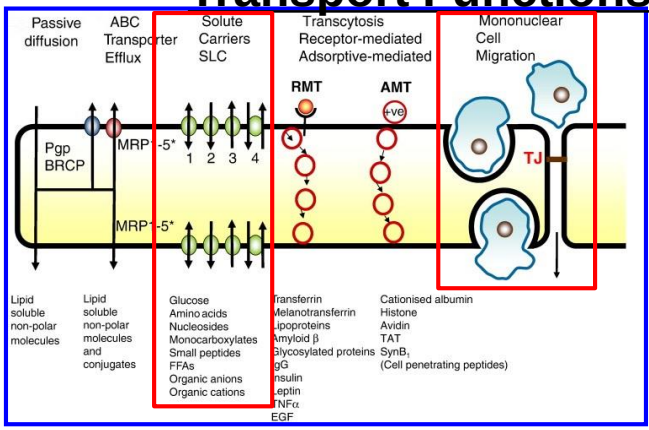
The Blood-Brain Barrier (BBB):
A specialized endothelium with physical barrier and transport functions

Paracellular Barriers

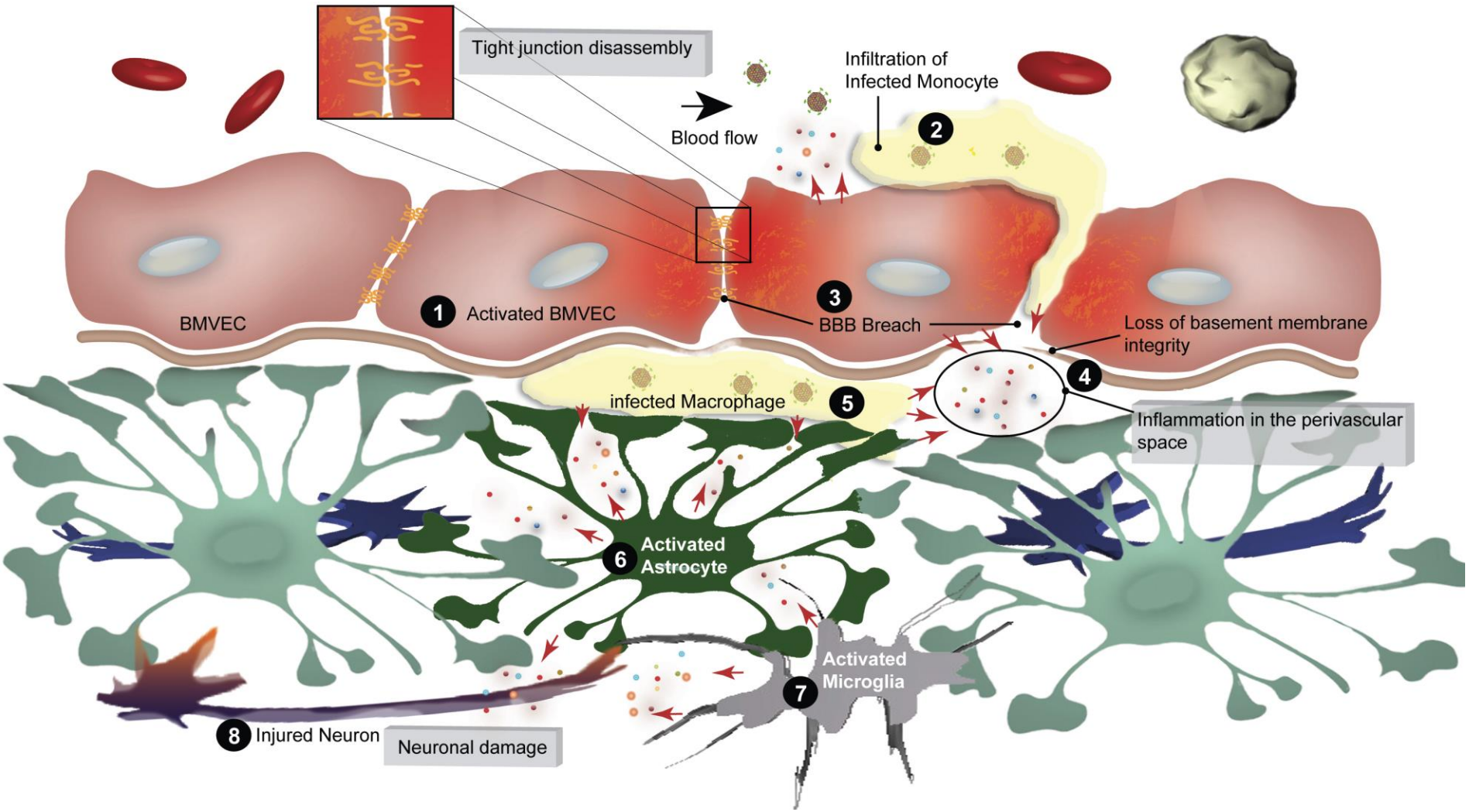




Transport Functions



Adapted from Abbott et al (2006) Nat Rev Neurosci 7: 41 &
Abbott et al (2010) Neurobiol Dis 37: 13.



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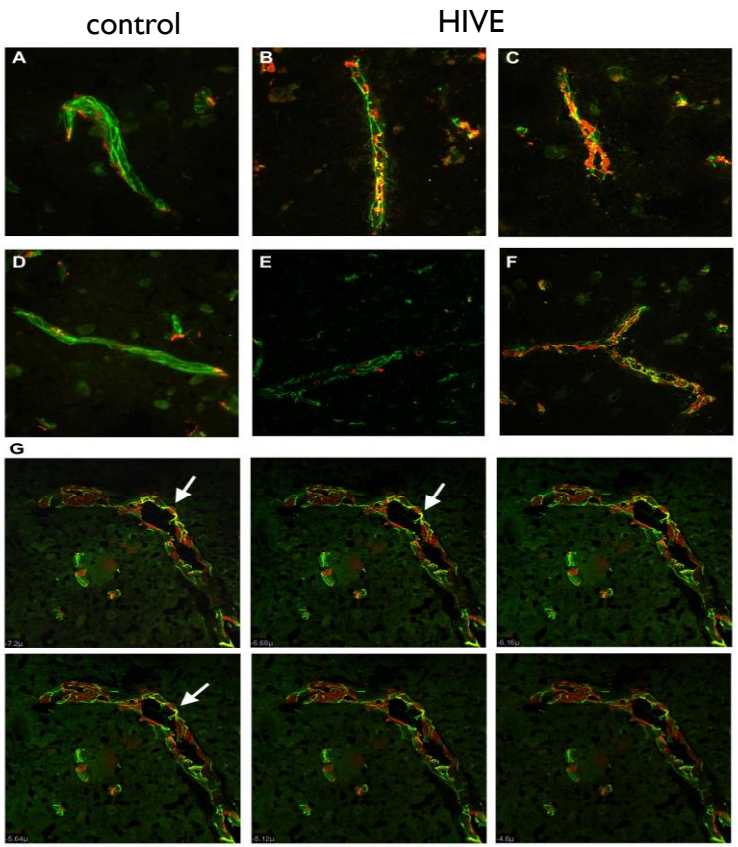
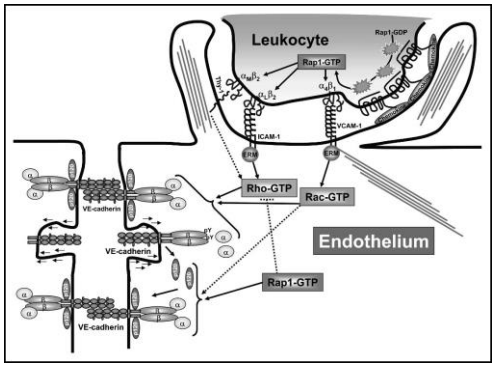
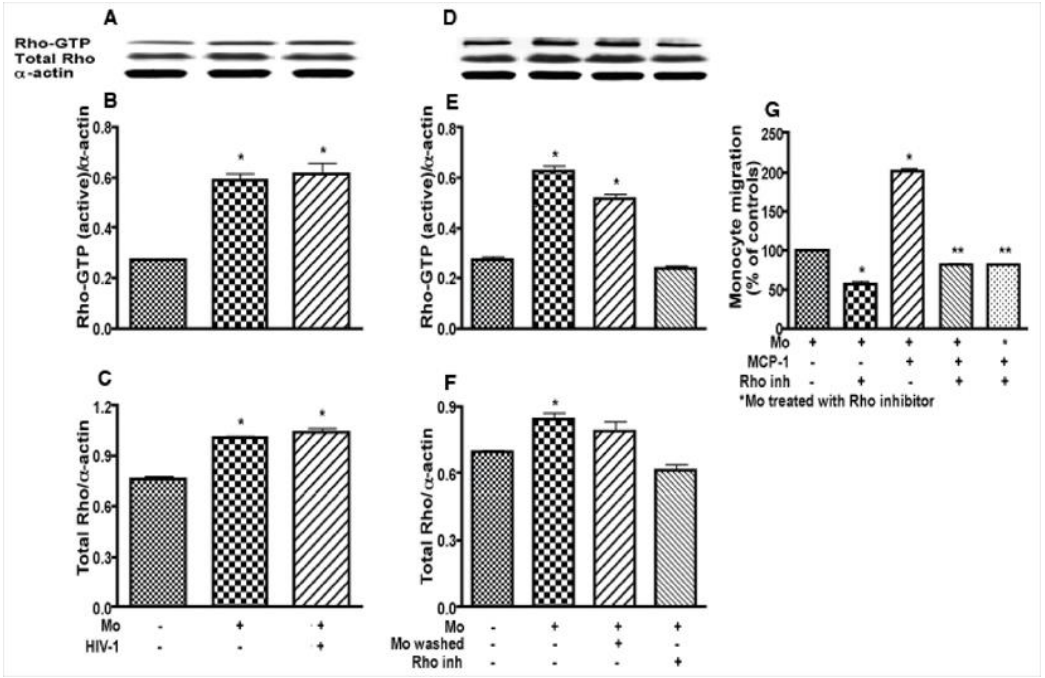
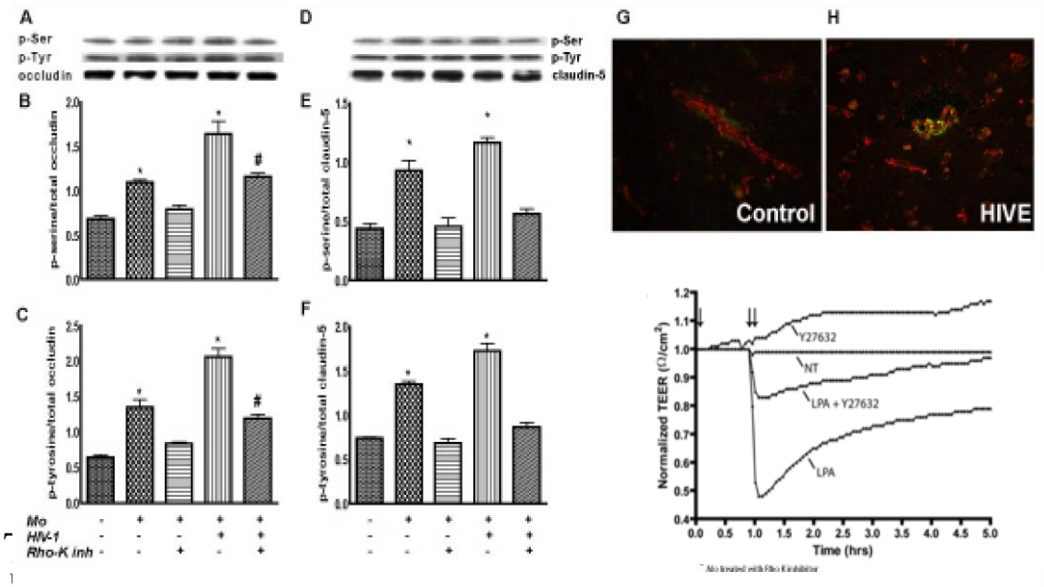
BBB compromise in HIV-1 encephalitis (HIVE): Tight junction (TJ) alterations regulated by Rho/Rho kinase activation during monocyte infiltration in brain

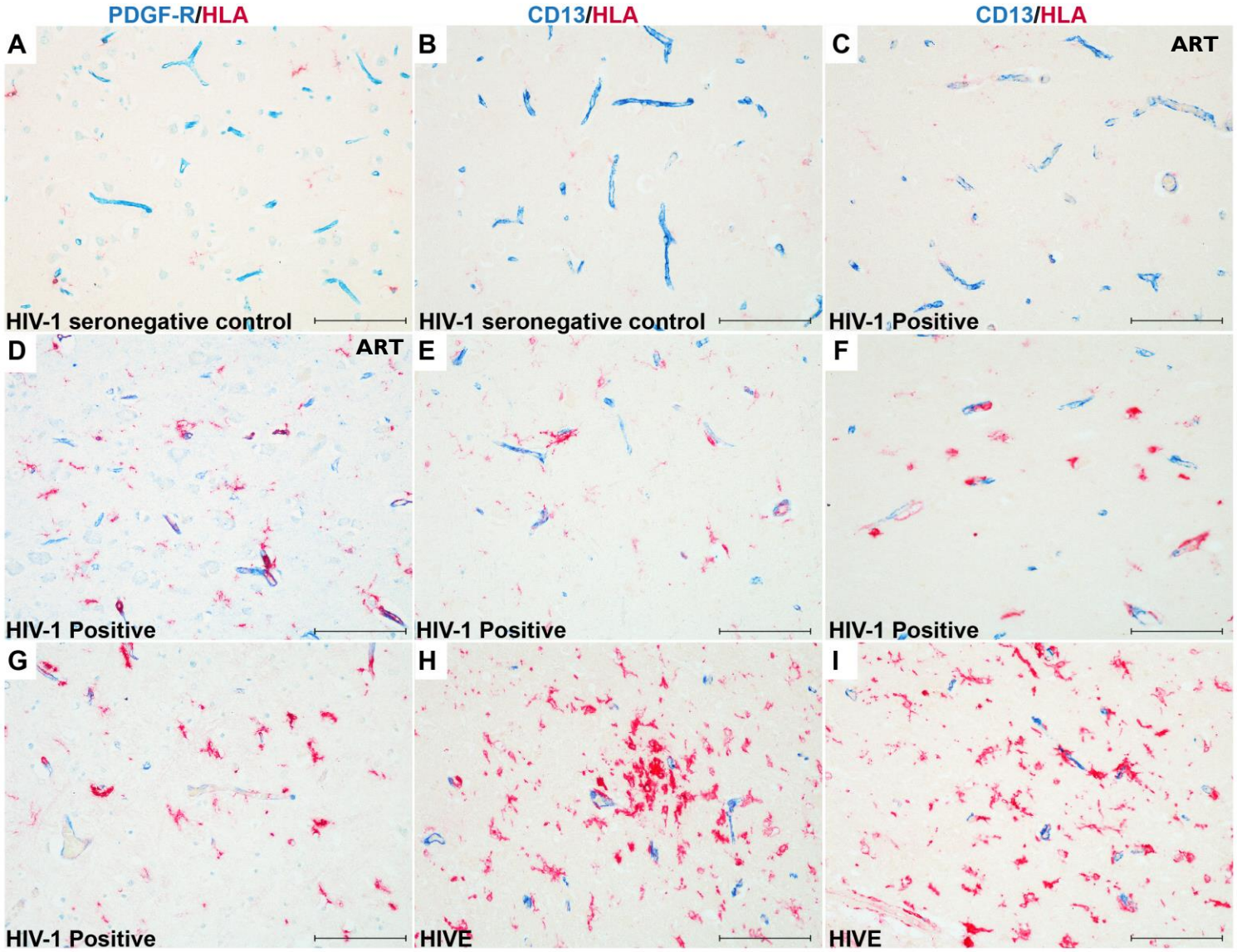


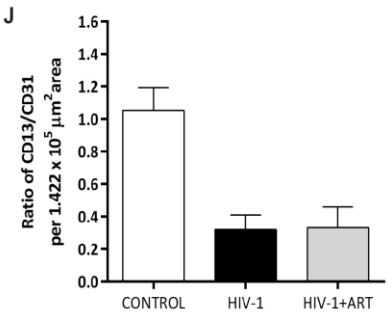






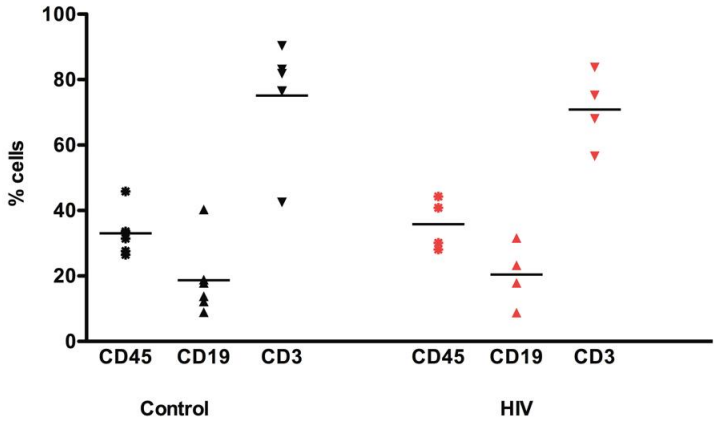
Diminished pericyte coverage of BBB in HIV-1-infected patients paralleling macrophage/ microglia activation

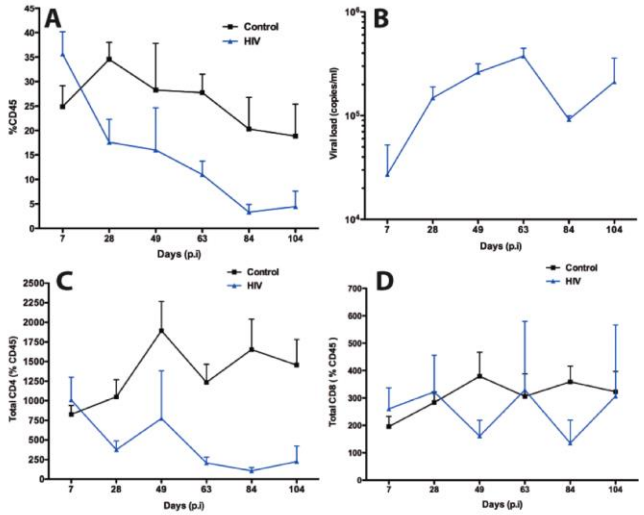


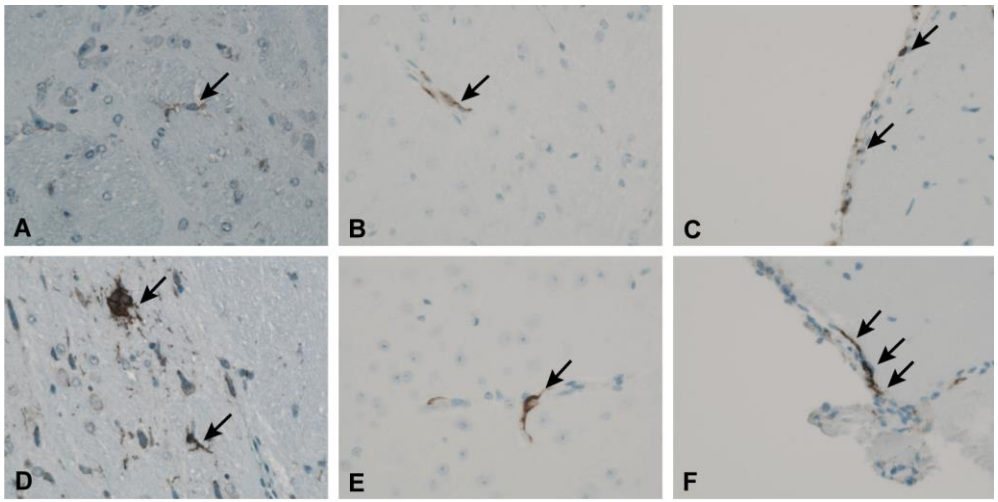


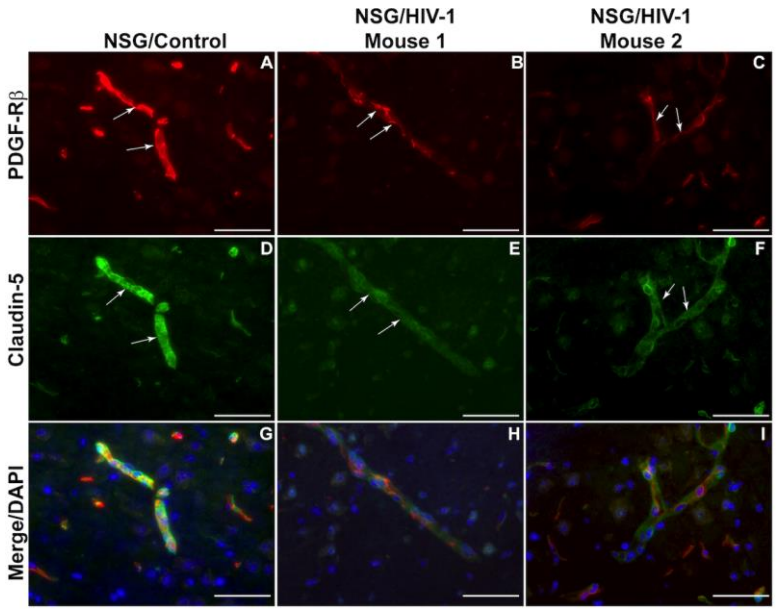
Persidsky et al. JCFM 20

HIV-1 infected humanized NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl} /SzJ, huNSG mice









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- Polydrug abuse (including smoking, opioids/other drugs, alcohol use disorder (AUD) presents significant but understudied problem, especially in patients living with HIV (PLWH).

- The high level of coincident tobacco use among intravenous drug abusers is noted in literature.

- A majority of heroin abusers are also tobacco smokers, and the smoking lengthens the duration of heroin reinforcement.

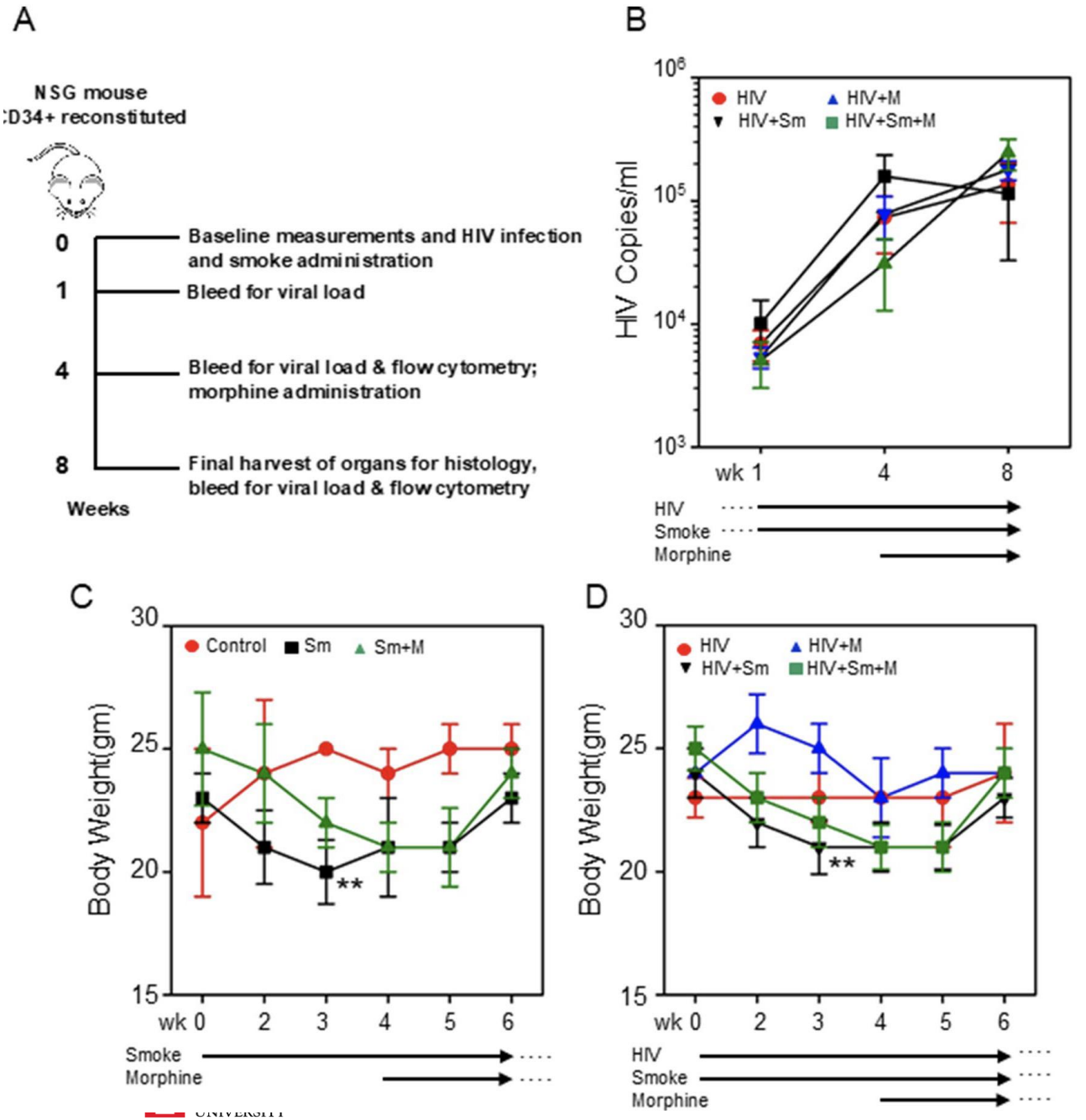
- Clinical data indicate that over 85% of methadone-supported patients use tobacco products.

- Smoking is 3-4 times more prevalent in PLWH and is considered major factor in cognitive decline in this population.

HIV viral load and body weight kinetics in smoke/morphine exposed NSG mice.

Morphine administration was conducted using 28 day Alzet mini-pumps (1 mg/kg/day)

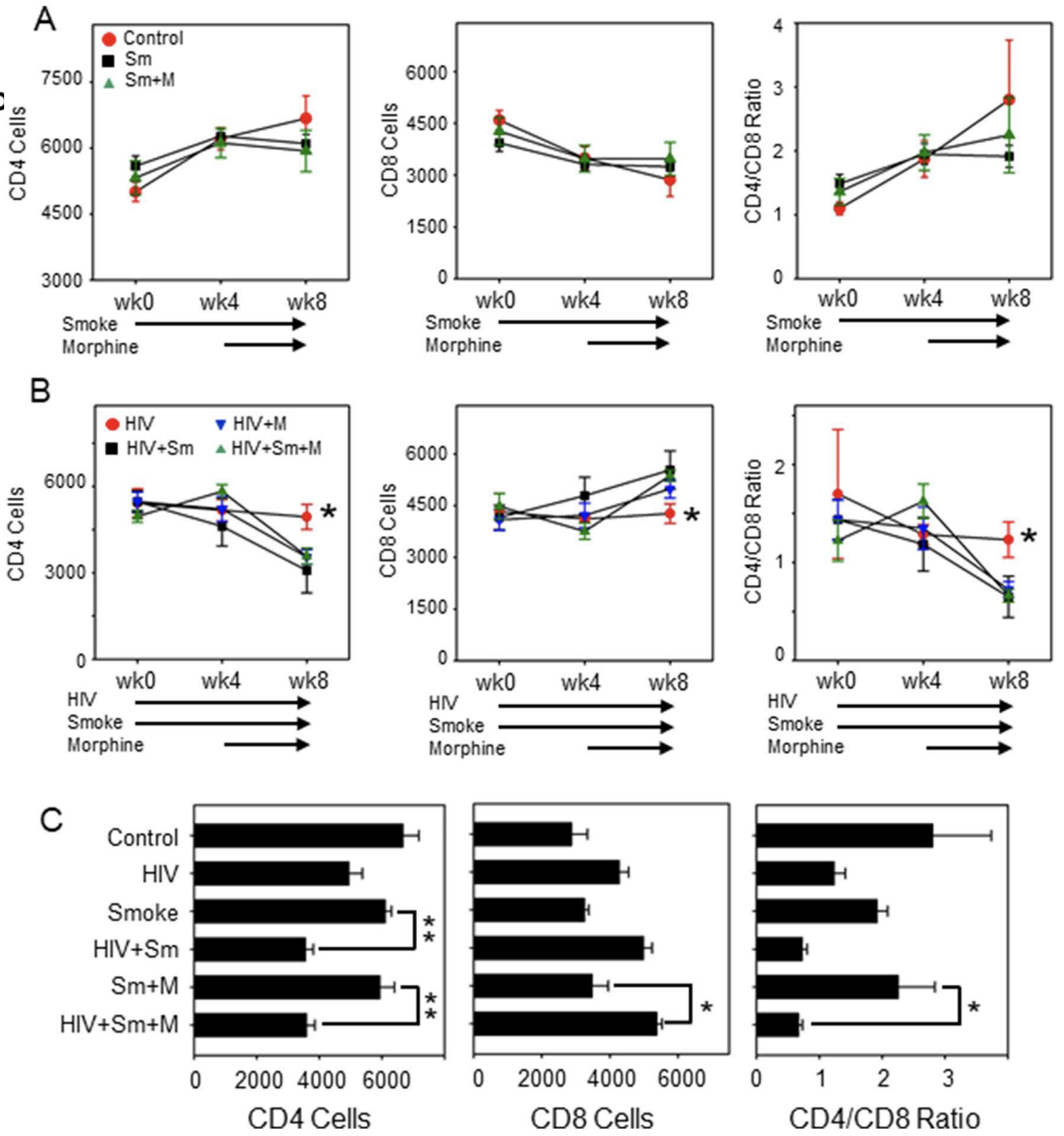
The morphine alone group did not survive the pump implants due to defective pumps



Cornwell et al Sci Rep 2020

Peripheral blood T cell profile in humanized mice exposed to smoke/morphine and infected with HIV

We observed a significant change in circulating CD4 and CD8 levels following either smoke exposure, chronic morphine administration, or the combination of these insults with HIV infection.



Cornwell et al Sci Rep 2020

Expression of PD-1, perforin and granzyme B in T cells

Neither smoke nor morphine exposure altered the expression of the T cell inhibitory receptor PD-1 in non-infected animals.

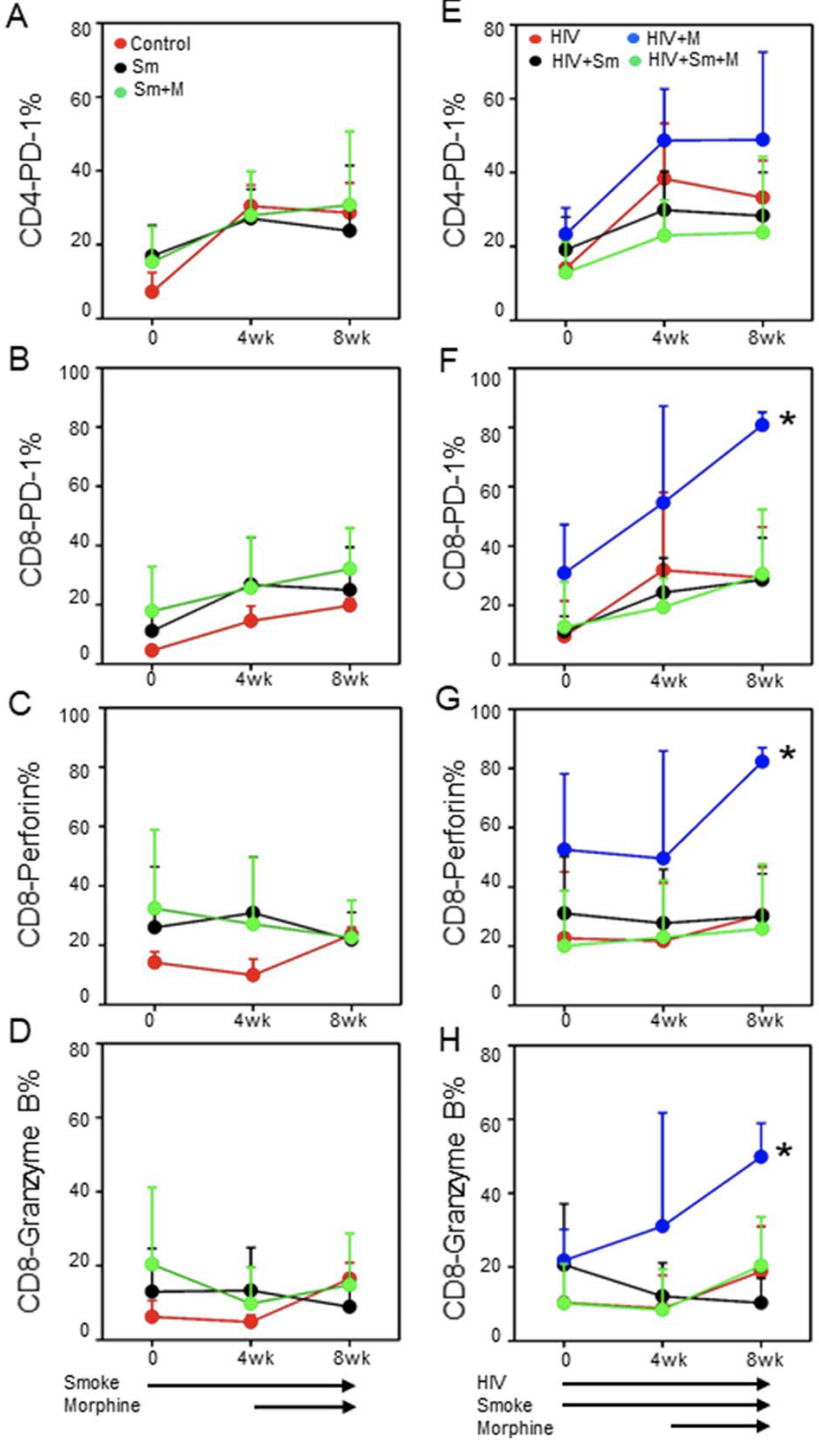
In HIV-infected animals, the expression of PD-1 by CD8+ T cells increased in the animals receiving chronic morphine administration.

Smoke exposure alone, and the combination of smoke and morphine, failed to increase the expression of PD-1.

Exposure to either smoke or the combination of smoke and morphine did not exert a significant effect on either perforin or granzyme B in CD8 T cells of uninfected animals. The expression of perforin or granzyme B was not altered by either smoke or the combination of smoke and morphine in CD8 T cells from HIV-infected animals.

The expression of perforin and granzyme B by CD8 T cells was significantly up-regulated following morphine administration by CD8 T cells from HIV-infected mice.

Cornwell et al Sci Rep 2020



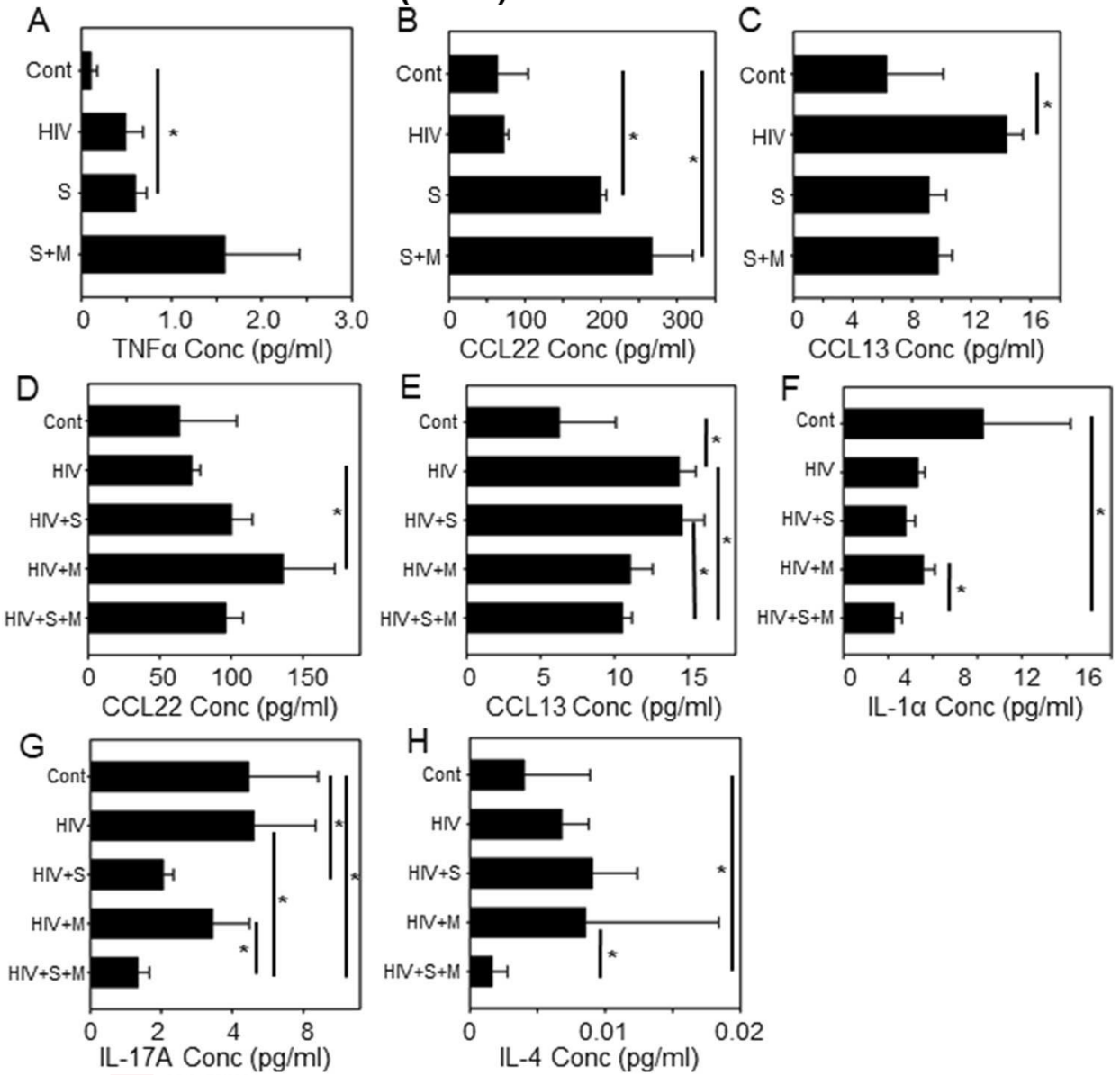
Human cytokine levels in animals exposed to either smoke or morphine in the plasma of NSG mice (A–C). Human cytokine levels present in the plasma of smoke and/or morphine- treated HIV-infected NSG mice (D–H).

We analyzed the levels of 29 cytokines and other biomarkers, and we found that only CCL13 was significantly elevated following HIV infection.

Exposure to smoke induced elevated the level of TNFα, and both smoke and the combination of smoke and chronic morphine up-regulated the level of CCL22 in non-infected animals.

The combination of smoke and morphine administration induced a significant reduction in the levels of IL-1α, IL-4 and IL-17A, while neither smoke nor morphine alone mediated the same inhibitory effect.

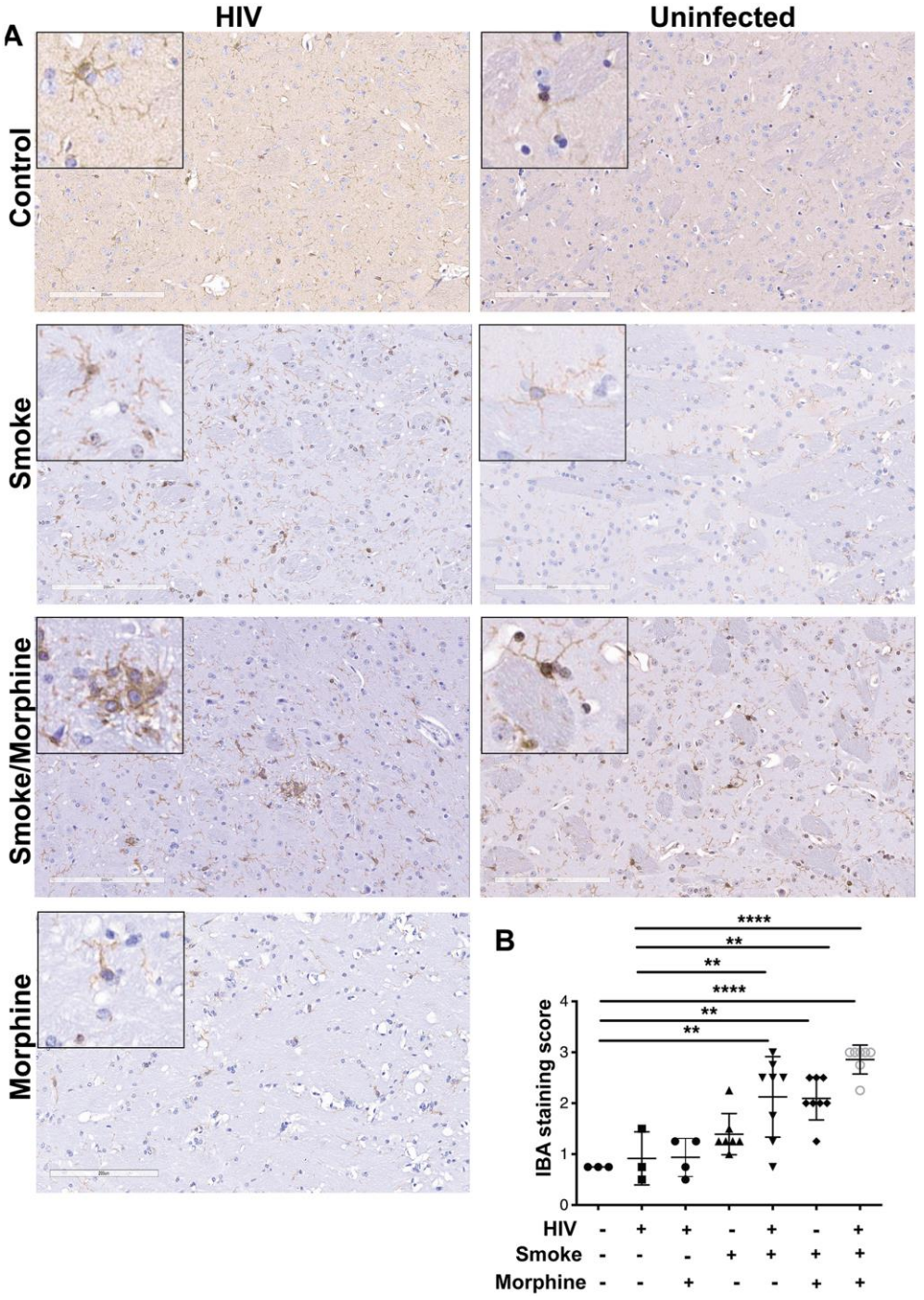
Cornwell et al Sci Rep 2020



Microglial activation in HIV-infected NSG mice exposed to morphine, smoke or a combination of HIV, morphine and smoke

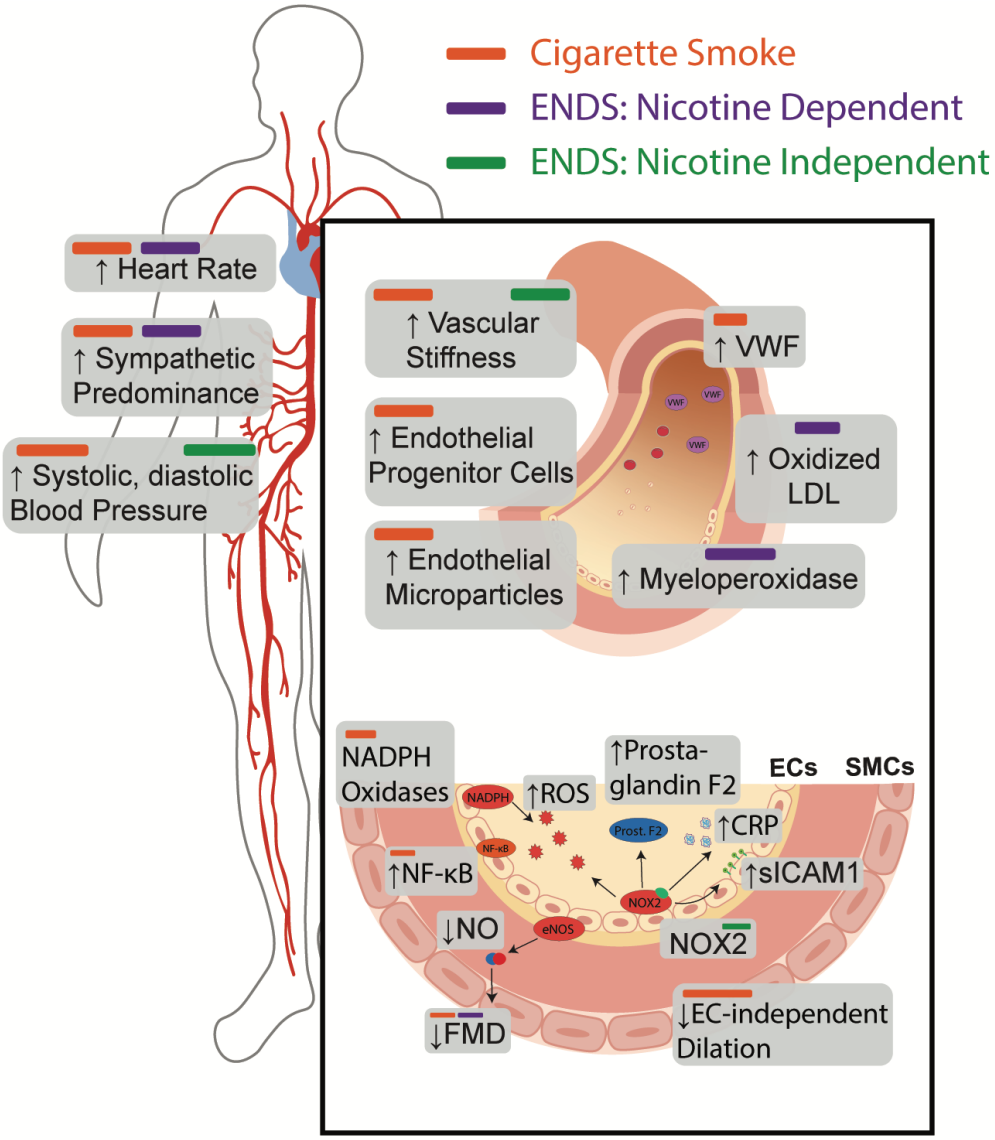
While smoke without HIV infection failed to significantly enhance IBA-1 expression, HIV-infected animals exposed to smoke showed a significant augmentation of microglial activation.

Both uninfected and HIV-infected animals treated with the combination of smoke and morphine exhibited a significant increase in the level of microglial activation.



Cornwell et al Sci Rep 2020

Electronic cigarettes (**e-cig**) health effects are driven by effects on microvasculature



During the last few years, electronic cigarettes (**e-cig**) have become very popular especially among adolescent users.
E-cig serve as nicotine delivering devices that have gained popularity due to a variety of flavors appealing to young users, aggressive marketing, and perceived safety as compared to combustible tobacco.

The health effects of e-cig remain unknown and very limited data indicate that they adversely affect immune responses, cause systemic endothelial cell dysfunction and result in a pro-inflammatory phenotype in macrophages /endothelial cells in the lung, leading to increased tissue injury during viral and bacterial Infections.
Increased amounts of cytokines, oxidative stress and inflammatory cells (neutrophils, macrophages) were found in lungs of chronically e-cig exposed animals

While being addictive, e-cig effects on the brain and cognition are essentially unknown.
Our data indicate that e-cig disrupt BBB function and increase macrophage accumulation in lungs, and enhance neuroinflammation.

Heldt et al Amer J Pathol 2021

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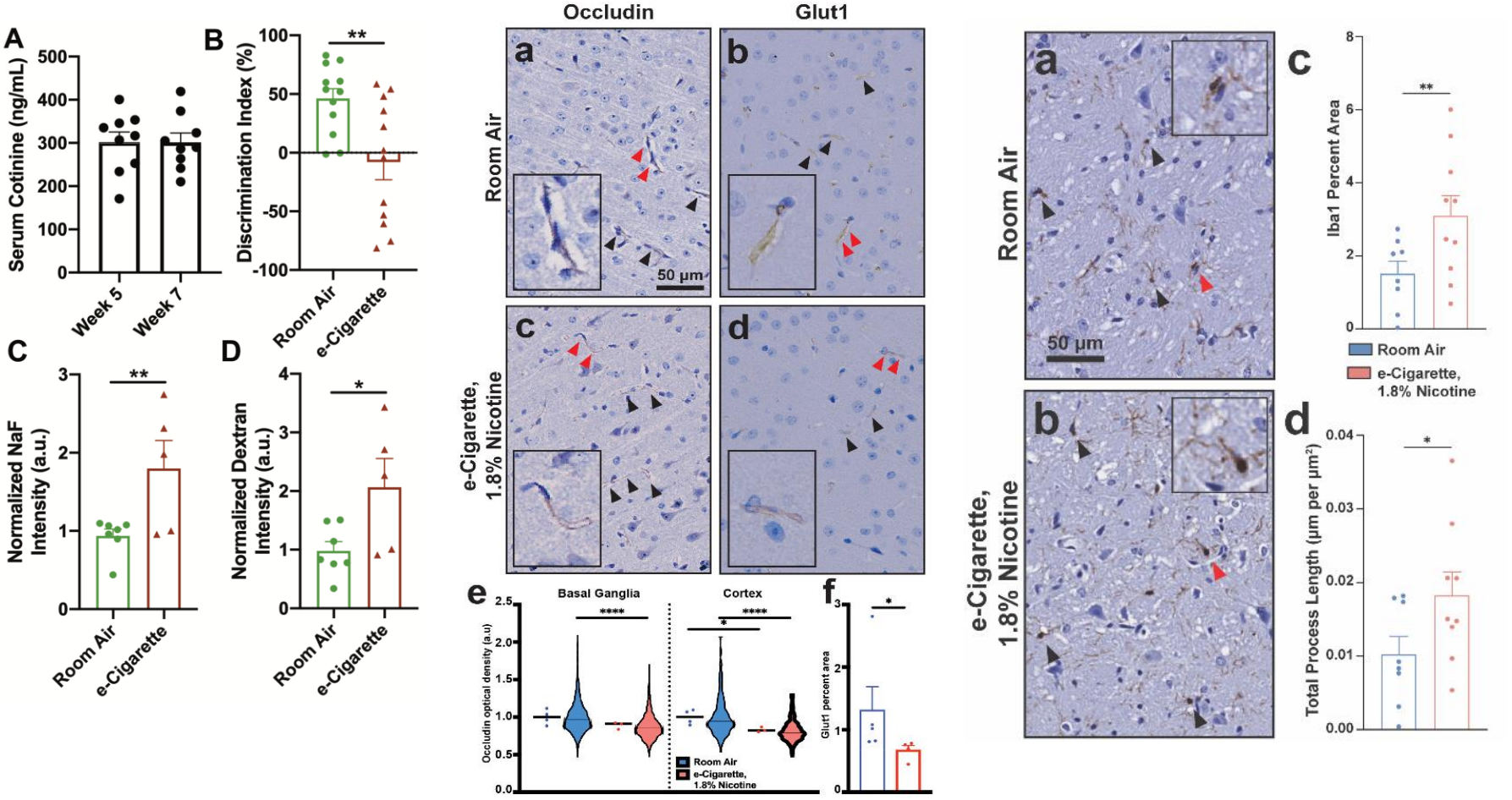
Increased BBB permeability, impaired memory decreased Occludin and Glut1 staining and enhanced microglia reaction in brains of mice exposed to chronic e-cig vapor



Heldt et al (2020) Brain Behav Immun

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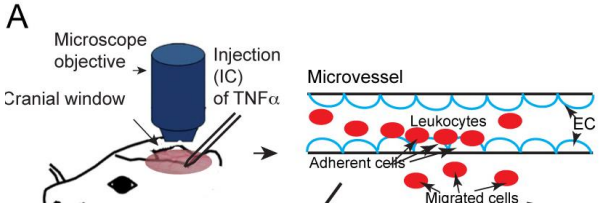
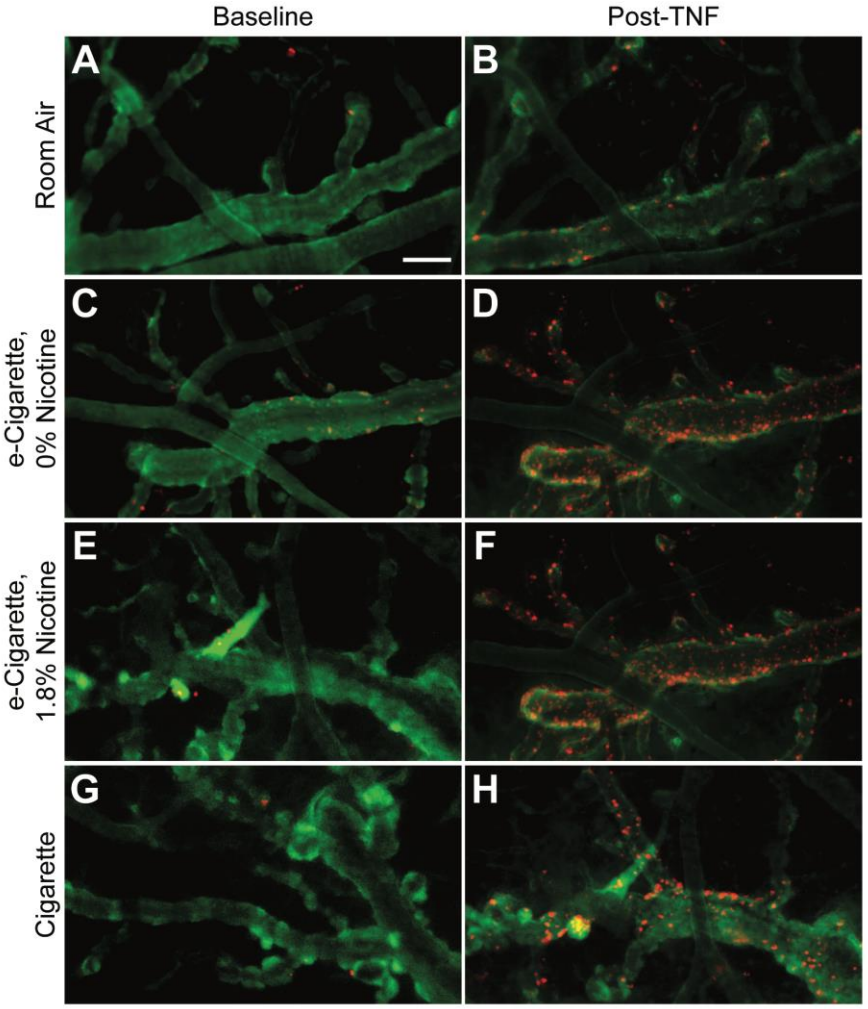
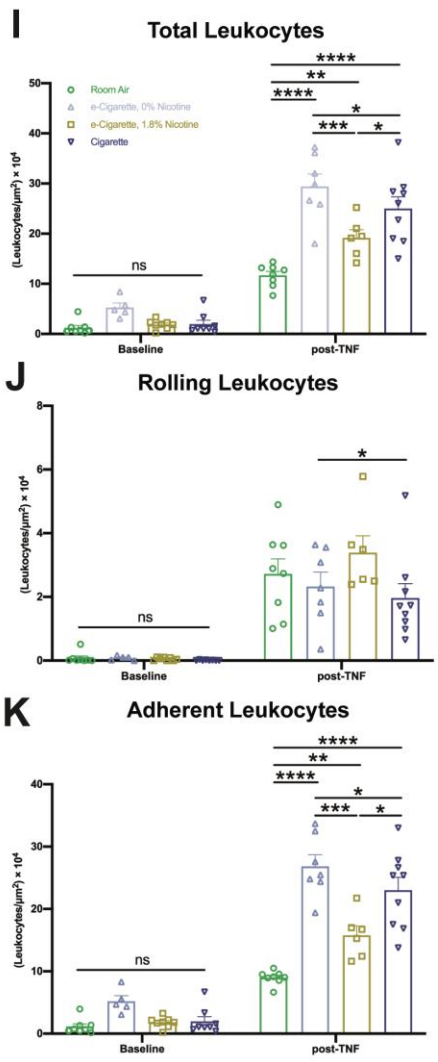
Chronic e-cig exposure enhances leukocyte adhesion to brain endothelium in vivo upregulated pro-inflammatory genes in brain endothelium







Cytokines/Inflammatory Signalling	
Cxcl10	1.93
Il6	0.56
Ccl2	1.71
Cd40	1.65
Tgfbr2	1.37
Tirap	1.76
Tnfsf12	1.23

Cell Adhesion Molecules	
Icam1	1.55
Itga5	1.42
Selp	4.55
Sele	5.73
Pecam1	1.46

Transcription Factors	
Irak4	1.31
Irf1	1.52
Nfatc3	1.65

Other	
Edn1	1.94
Nox1	0.47
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Heldt et al (2020) Brain Behav Immun

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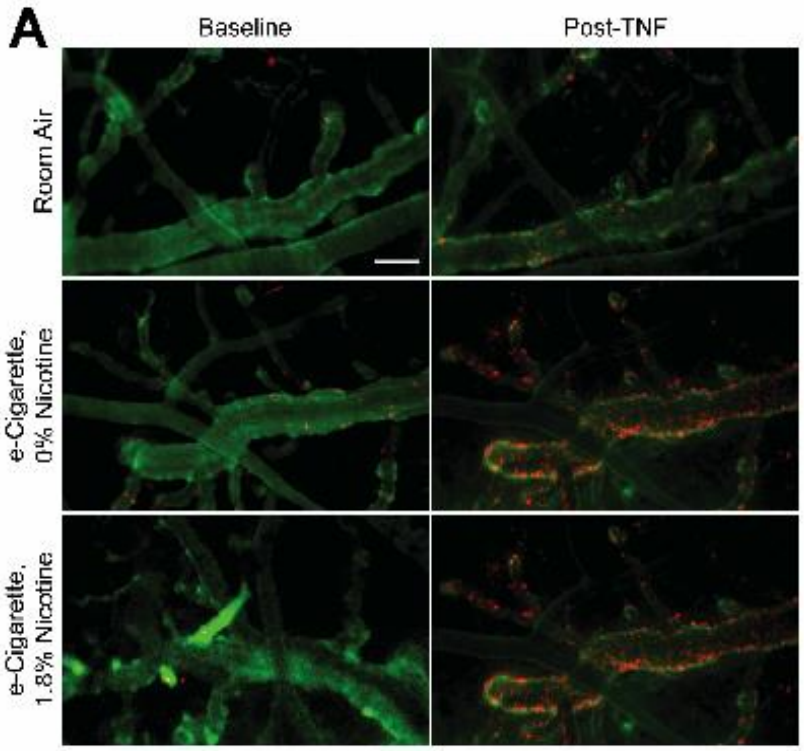

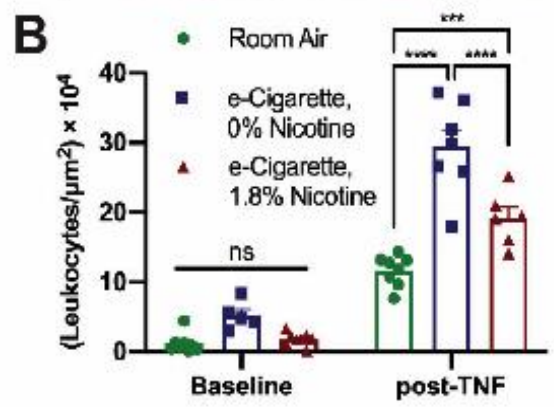

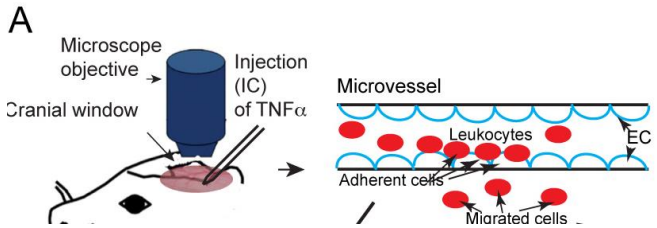

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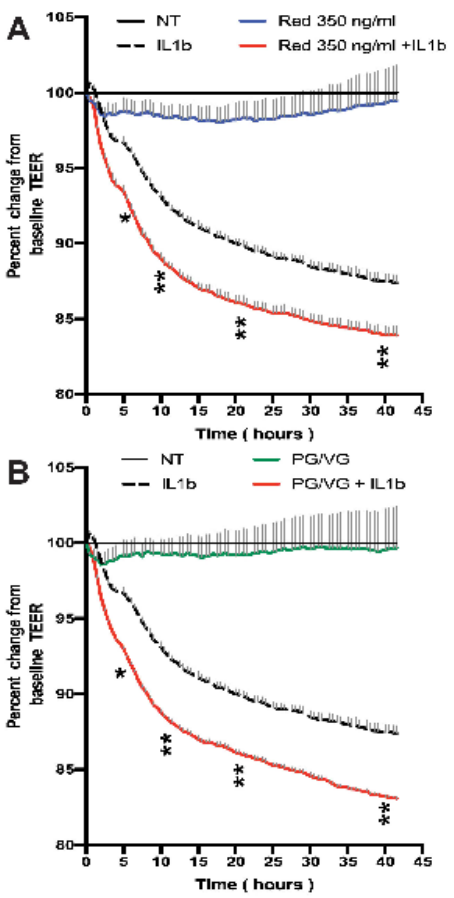
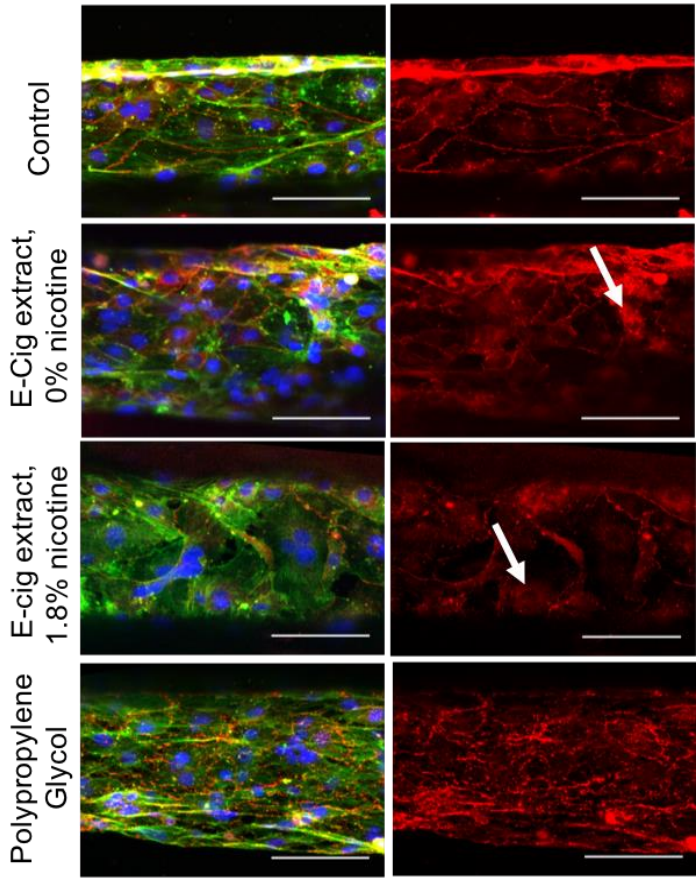
Heldt et al (2020) Brain Behav Immun

E-cig conditioned media diminished barrier integrity, and caused tight junction redistribution in e-cig exposed 3D models of BBB *in vitro*





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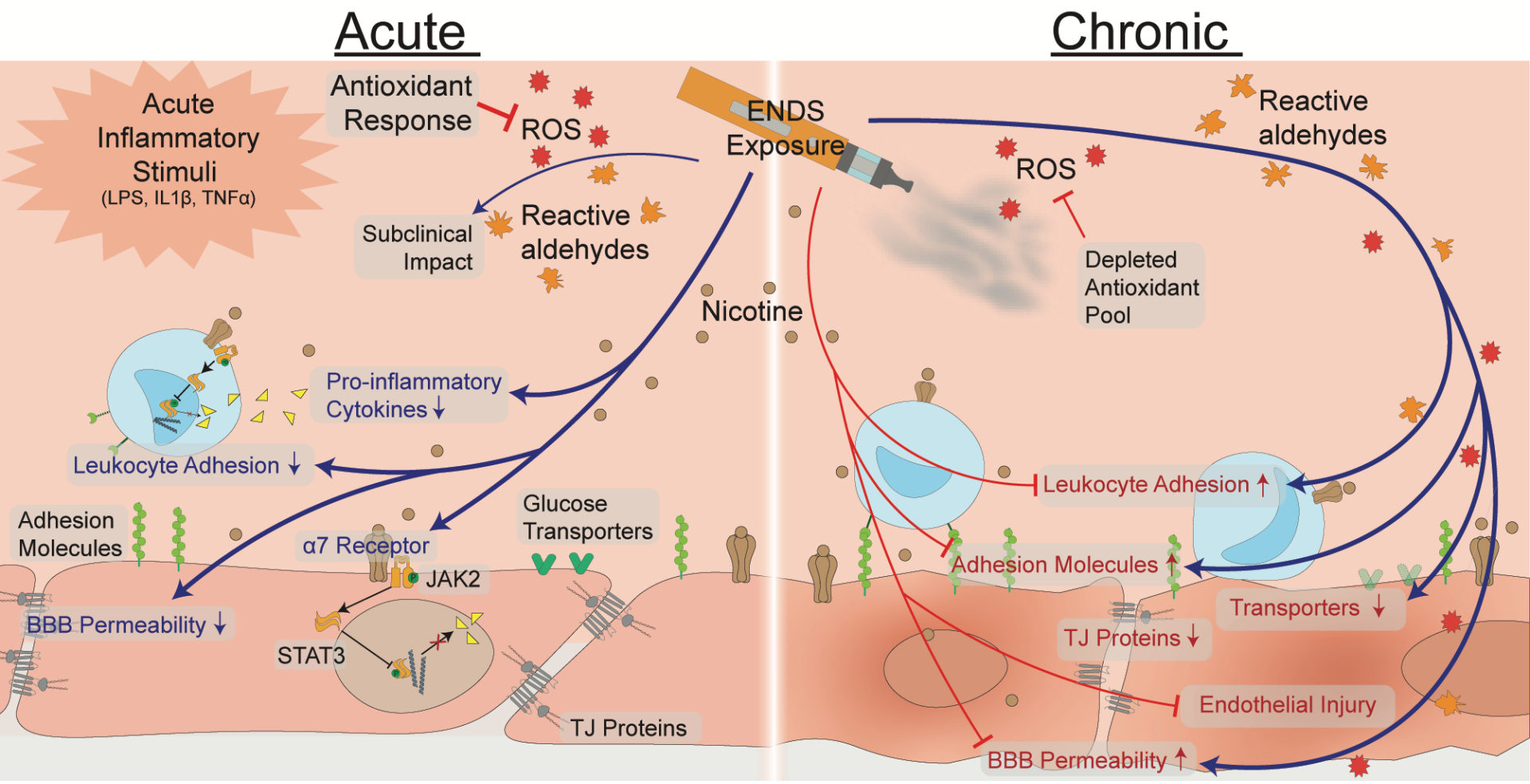
Effects of e-cigarettes on BBB



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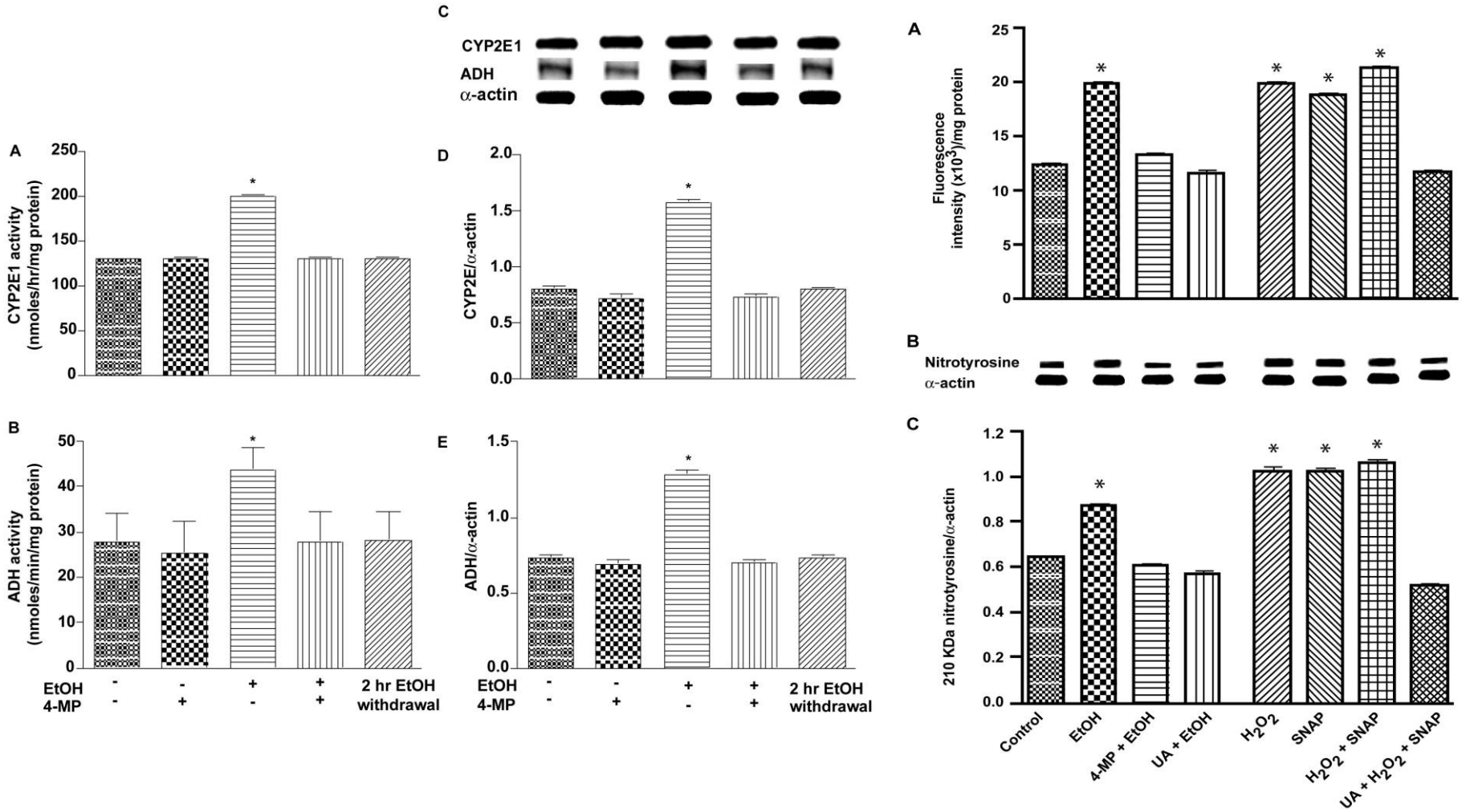
Heldt et al Amer J Pathol 2021

Why to study effects of alcohol abuse at blood brain barrier insetting of HIV infection?

- Combination of heavy drinking and HIV infection leads more significant cognitive impairment and structural brain abnormalities
- Effects of alcohol abuse on structural integrity of BBB could accelerate such abnormalities
- What are mechanisms of BBB injury by alcohol?
- Altered permeability of BBB and enhanced effects of alcohol in brain
- Protective strategies based on BBB shielding

Alcohol metabolism by CYP2E1 and ADH in brain endothelial cells results in oxidative stress



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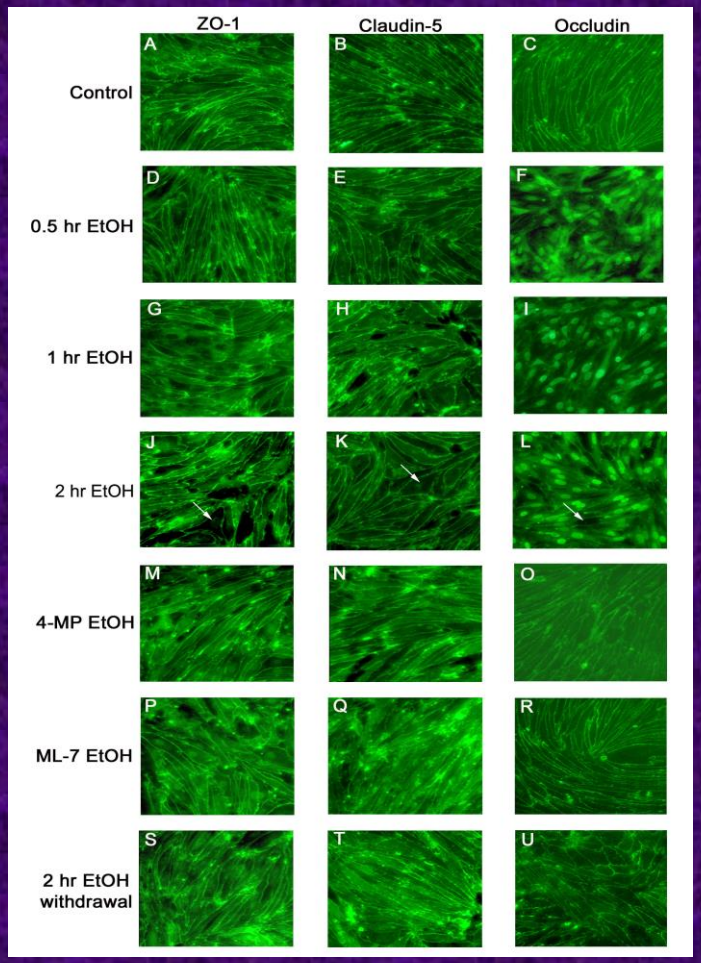
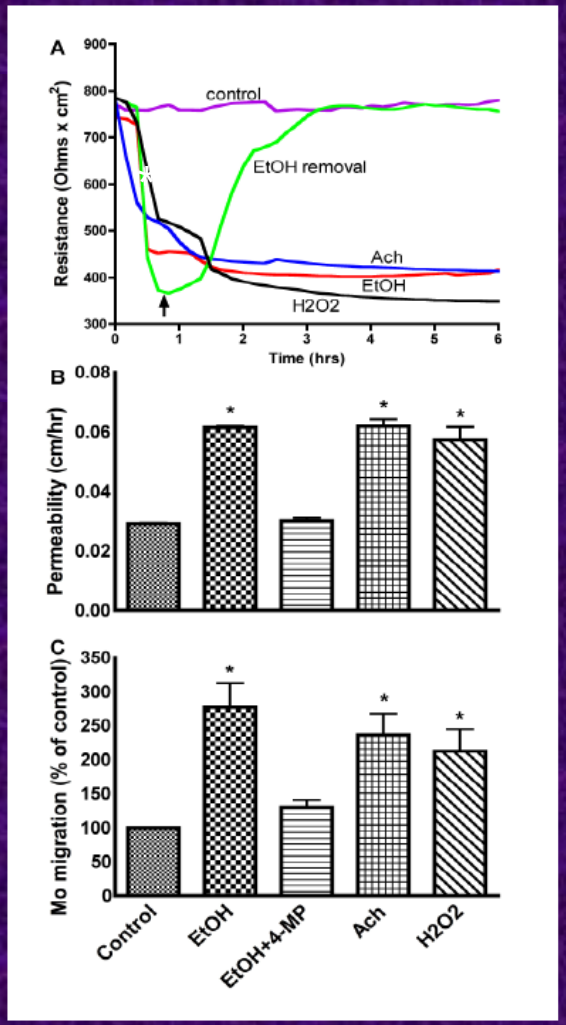
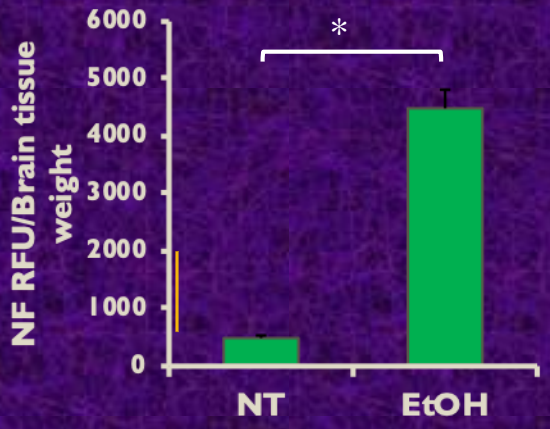
Haorah et al., Alcohol 2005

Effects of alcohol on transendothelial resistance, permeability and monocytes migration across brain microvascular endothelial monolayers *in vitro* and BBB permeability *in vivo*







EtOH concentrations (25-50 mM) used in this study are similar to ones seen in the peripheral blood of moderately to severely intoxicated humans, ≈1.5-3 times of legal limit (Deutch et al., 2004: Cherpitel et al., 2003).

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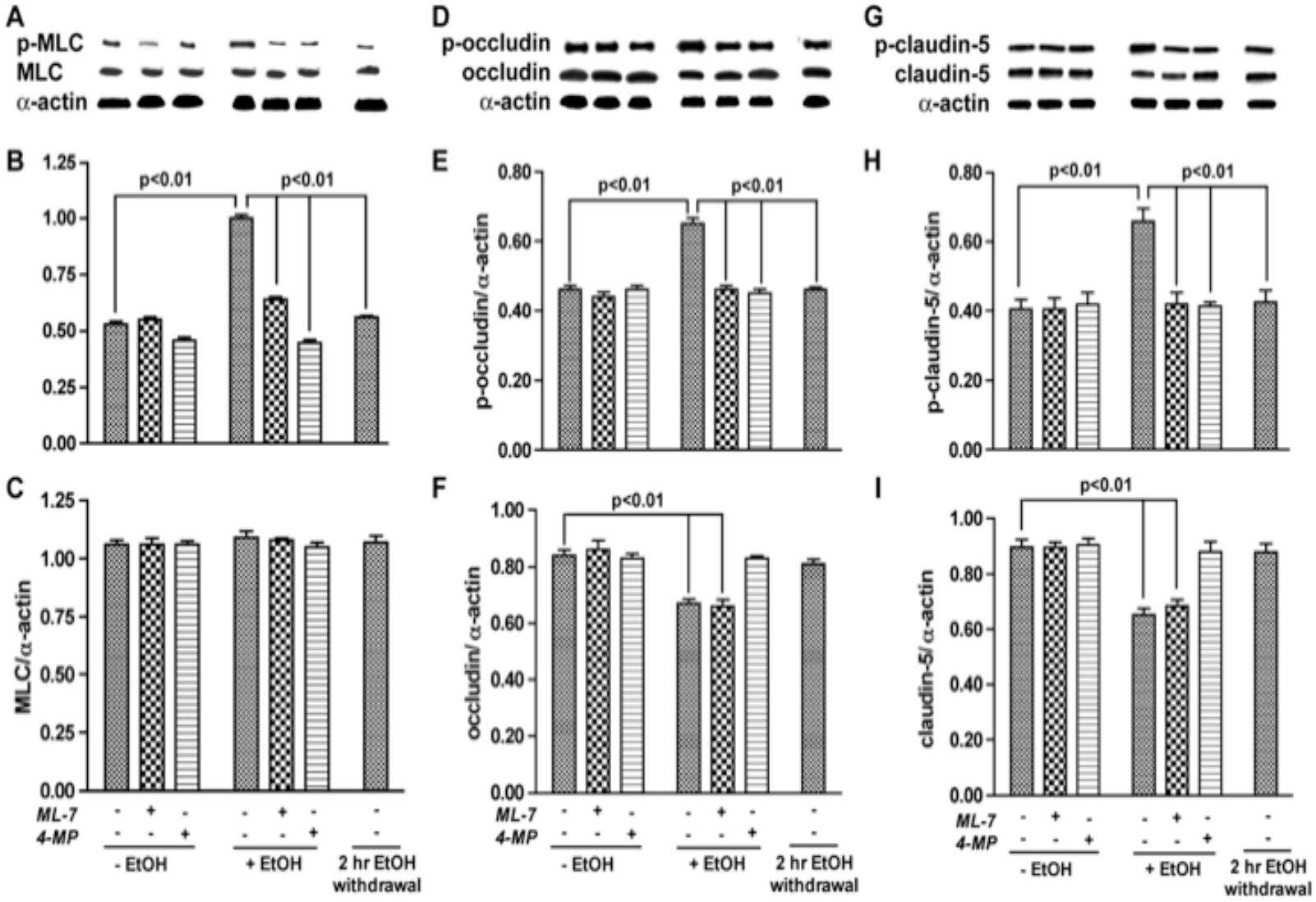
Haorah et al., 2005, 2007, 2008

Alcohol effect on myosin light chain (MLC) and TJ phosphorylation/content in BMVEC



Haorah et al., Leuk Biol 2005

Alcohol effects in hu-PBL-NOD/SCID mouse model for HIVE

HIV-1 infected hu MDM (day 0)

Human PBL (day -7)

Anti-asialo GM 1 (days-10, -5)

Anti murine - CD 122 (anti-LR-2R) (day -8)

Day 0 — EtOH — (Week 1) — (Week 2)

4%

or control liquid diet

Peripheral blood

HIV-1 p24 ELISA/Western blot for Ab

FACS

Sacrifice

Spleen - FACS

Brain - Quantitative neuropathology

Western blot

EtOH-mediated immunosuppression manifested by a significant decrease in CD8/IFNγ lymphocytes, a five-fold increase in viremia, and diminished expression of immunoproteasomes in the spleen.

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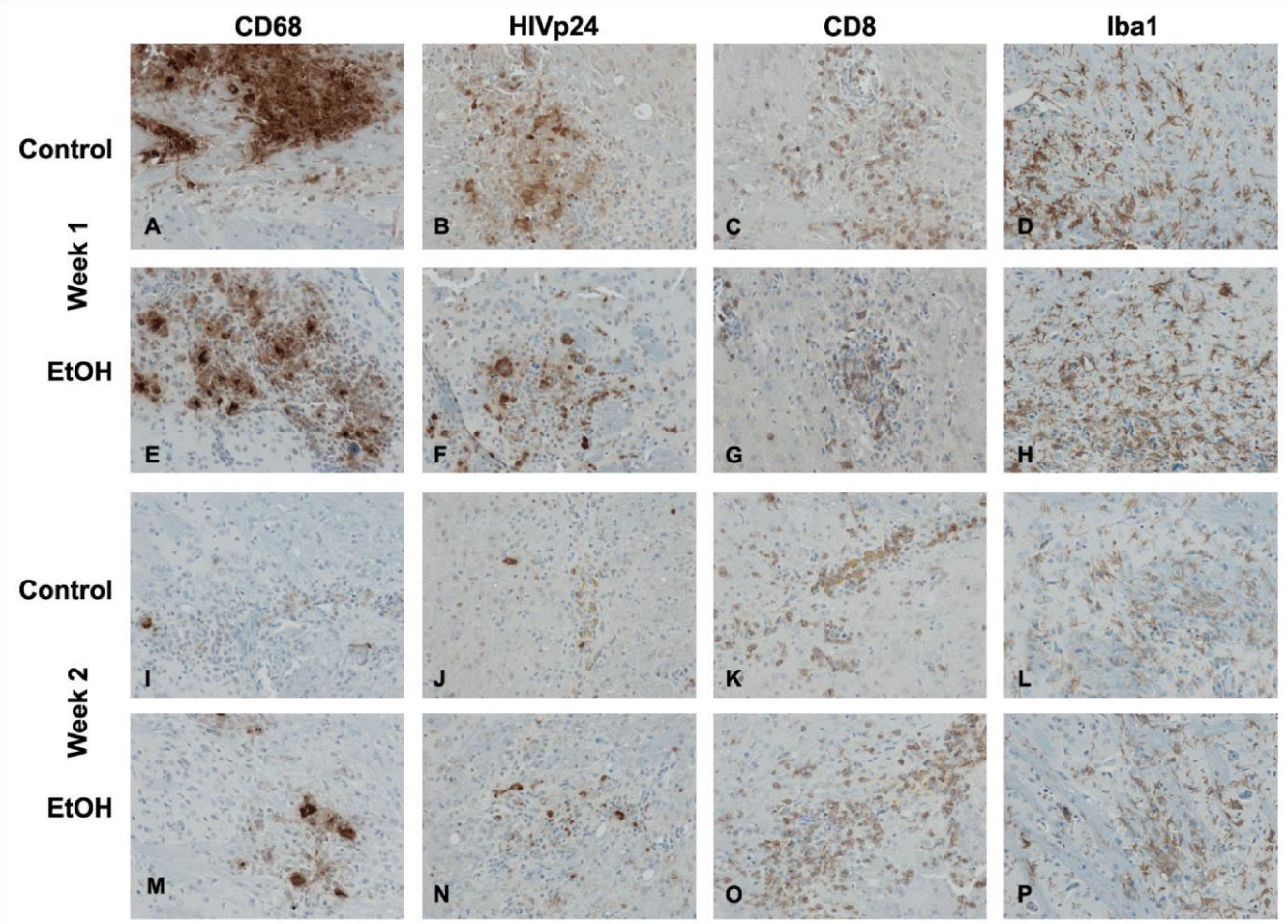
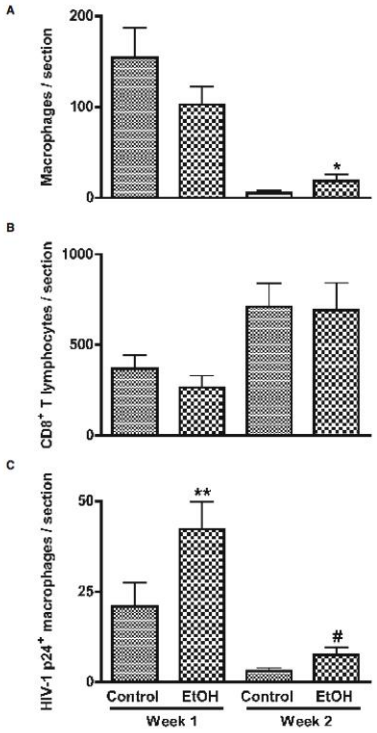
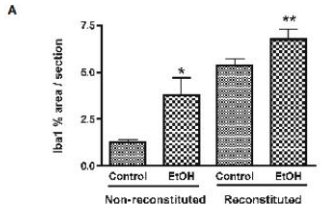
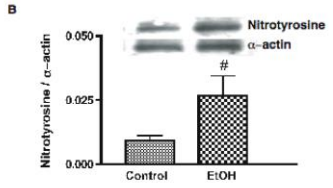
Neuro-inflammatory responses in alcohol-fed hu-PBL-NOD/SCID HIVE mice









Potula et al, 2006, Am J Pathol

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Inflammation as the cause of alcohol-induced neurodegeneration

Chronic alcohol consumption is associated with increases in serum proinflammatory cytokines

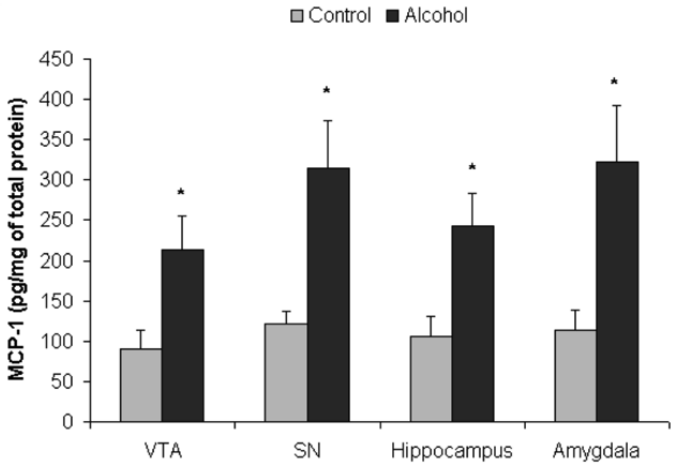
Monocytes isolated from the blood of alcoholics produce greater amounts of TNFα spontaneously and in response to endotoxin.

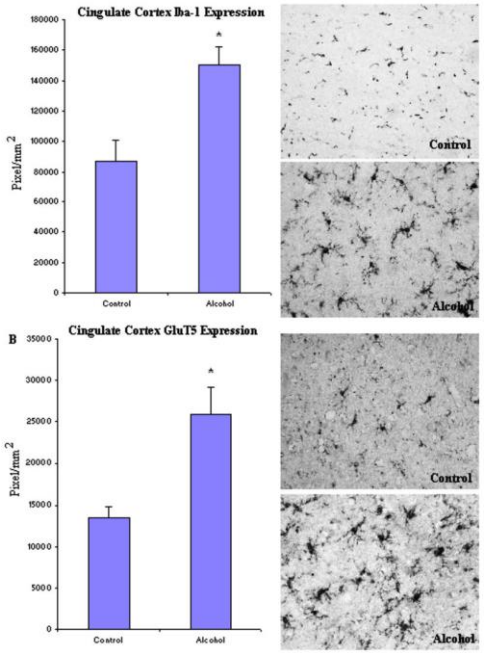
In the brains of chronic alcoholics microglial activation coincided with enhanced CCL2 production.

In vivo chronic EtOH administration followed by secondary pro-inflammatory stimulus (LPS) resulted in sustained up-regulation of cytokines (TNFα, CCL2, IL-1β) in the brain for long period of time.

Activation of brain microglia and increased brain expression of COX-2 and gp91phox NOX subunit mRNA were found in the EtOH-pretreated LPS group.

Combined alcohol and LPS exposure inhibited neurogenesis.





He and Crews Exp Neurol. 2008; 210(2): 349–358.

Agonists of cannabinoid receptor 2 (CB_2) possess potent anti-inflammatory properties and are devoid of the psychoactive effects of CB1R stimulators.

CB_2 are found predominantly on immune cells. CB_2 were also detected in brain (microglia and perivascular macrophages) and they are only detectable under in neuroinflammatory conditions

CB2R agonists may decrease of immune cell activation, and diminished secretion of pro-inflammatory factors by lymphocytes, macrophages and microglia

Cannabinoids have been reported to inhibit chemokine-induced chemotaxis of various cell types including neutrophils, lymphocytes, macrophages, monocytes and microglia. Mechanisms of anti-inflammatory effects mediated by CB_2 activation are not clear.

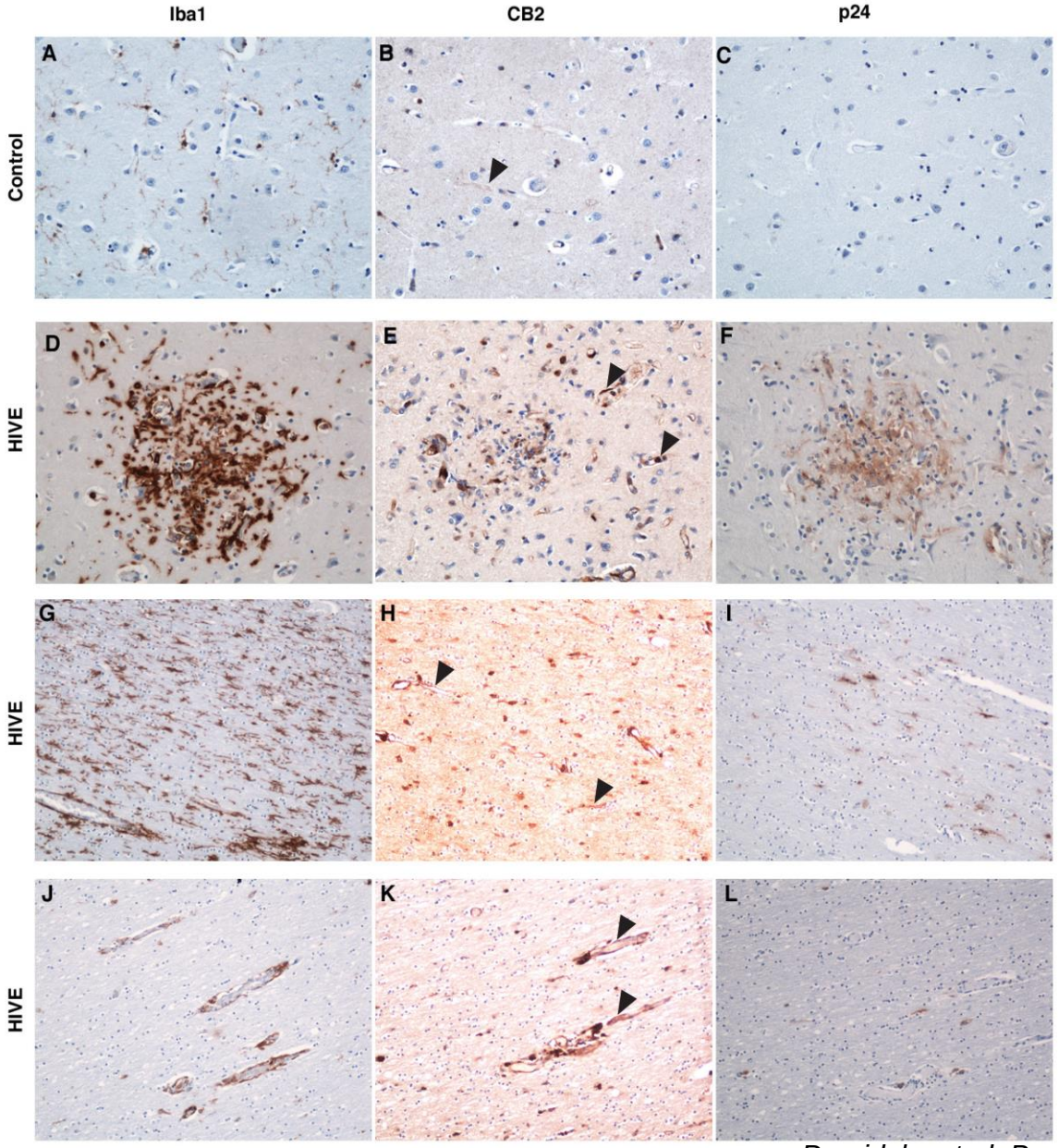
Anti-inflammatory properties of CB_2 agonists may be related to their actions on the endothelium.

Synthetic CB_2 agonists reduced TNFα-induced activation of human coronary artery endothelial cells *in vitro*

CB_2 activation afforded neuroprotection in animal models of stroke, multiple sclerosis and Alzheimer's disease.

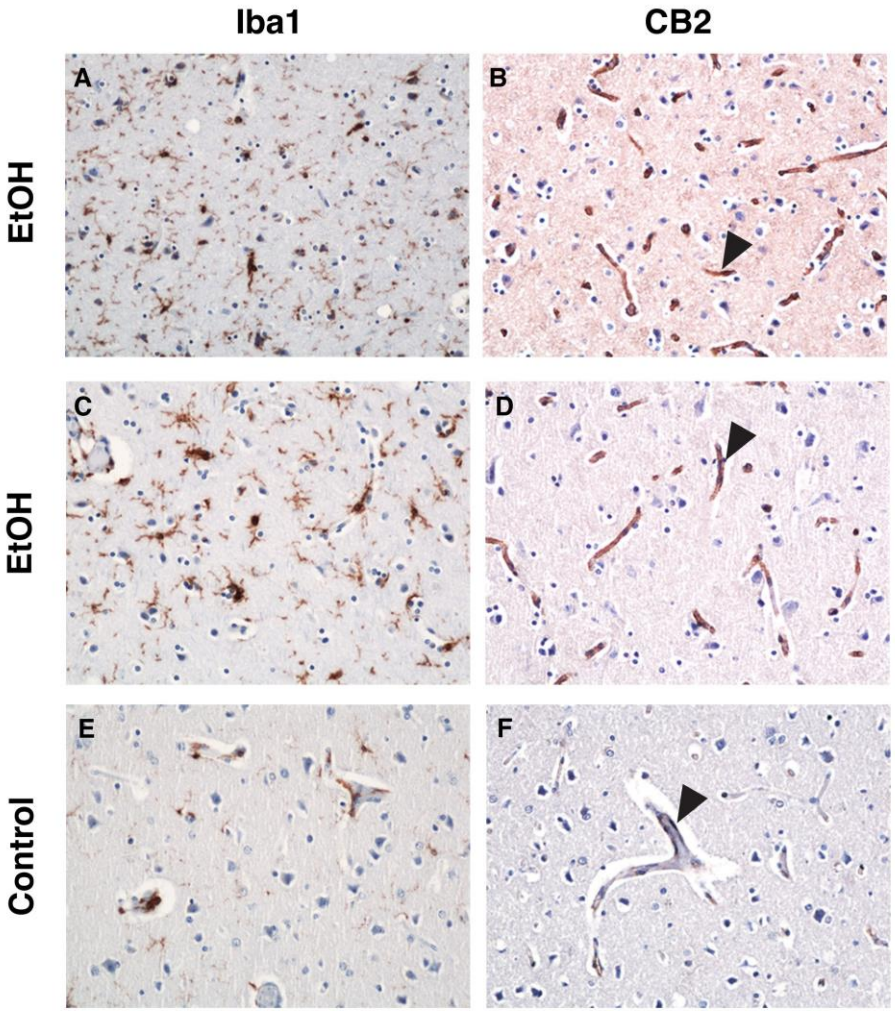
We propose that CB_2 activation will attenuate neurodegeneration and BBB injury caused by neuro-infammation and HIV-1 CNS infection via effects on monocytes, brain endothelium, activated microglia and HIV-1 infected macrophages

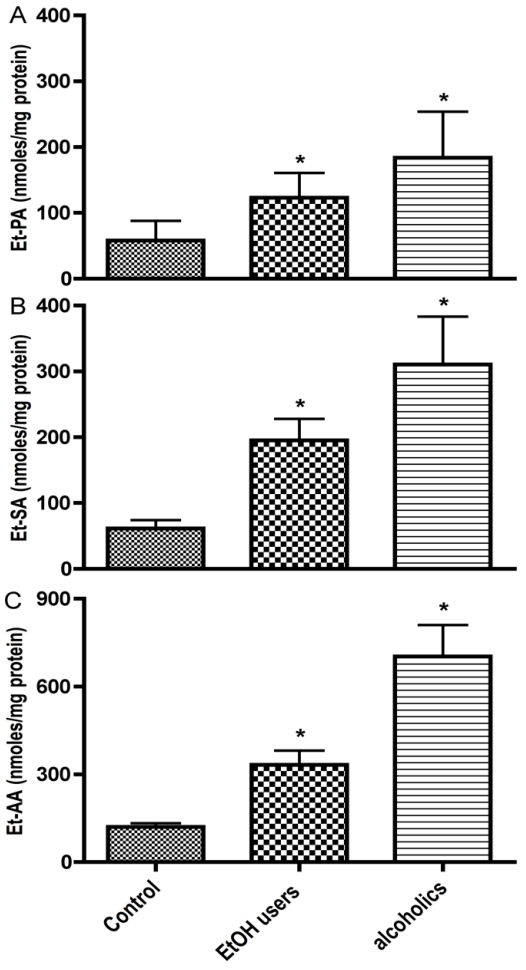
Up-regulation of CB_2 expression in microvessels and macrophages/microglia in HIV-1 encephalitis (HIVE)



Persidsky et al. Brain, Behavior Immunity 2011

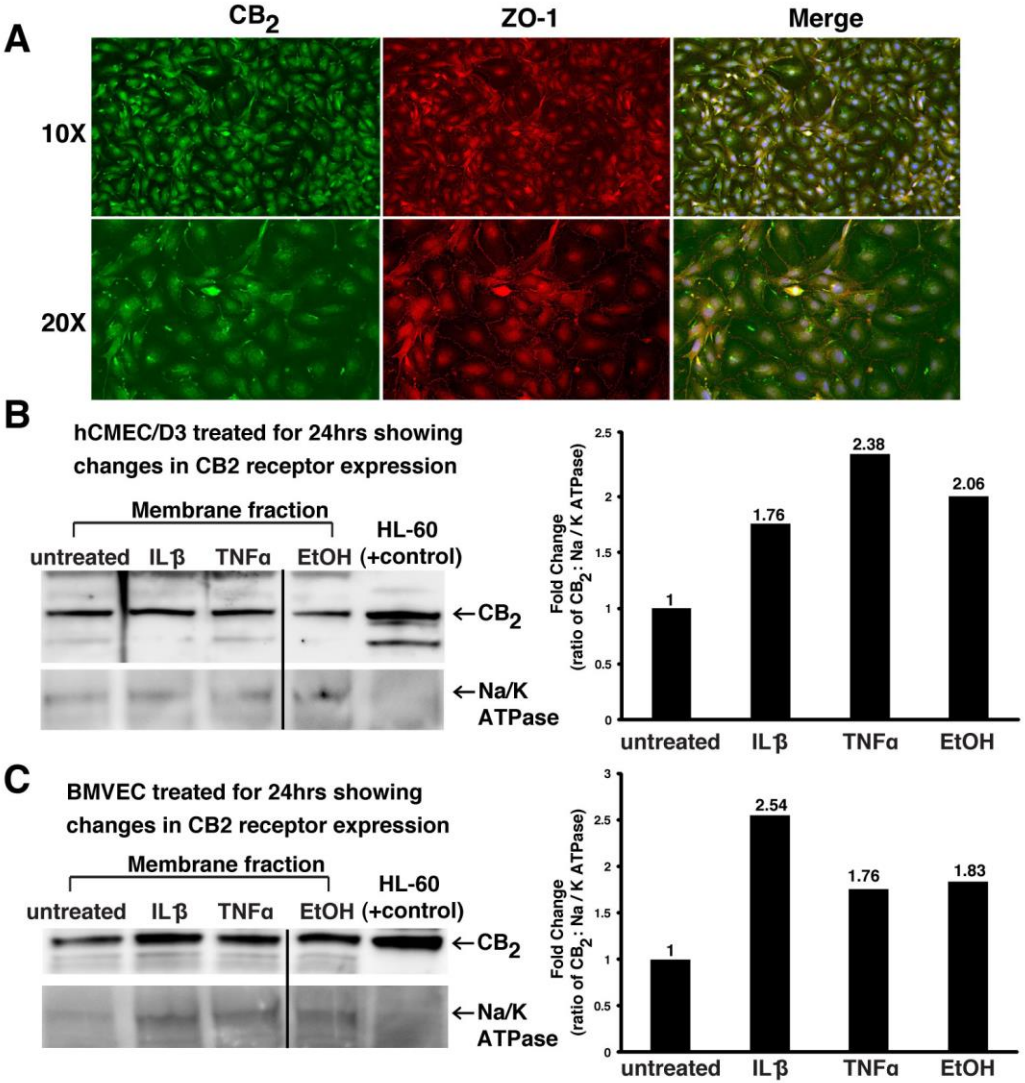
Up-regulation of CB_2 expression in microvessels of brain tissue of alcoholics





Fatty Acid Ethyl Esters FAEE (Et-PA, Et-SA, and Et-AA) levels were detected in post-mortem brain tissue extracts from alcohol users (very mild drinkers), alcoholics (chronic alcohol abusers and alcohol dependent subjects), and matched controls by GC-MS method. After SPE and column purification, 4 μl sample from each condition was injected onto GC equipped with a mass selective detector and analyzed for (A) Et-PA (B) Et-SA, and (C) Et-AA. Results were expressed as mean nmoles/mg protein ± STDEV (n = 7).

CB_2 expression in human brain endothelial cells



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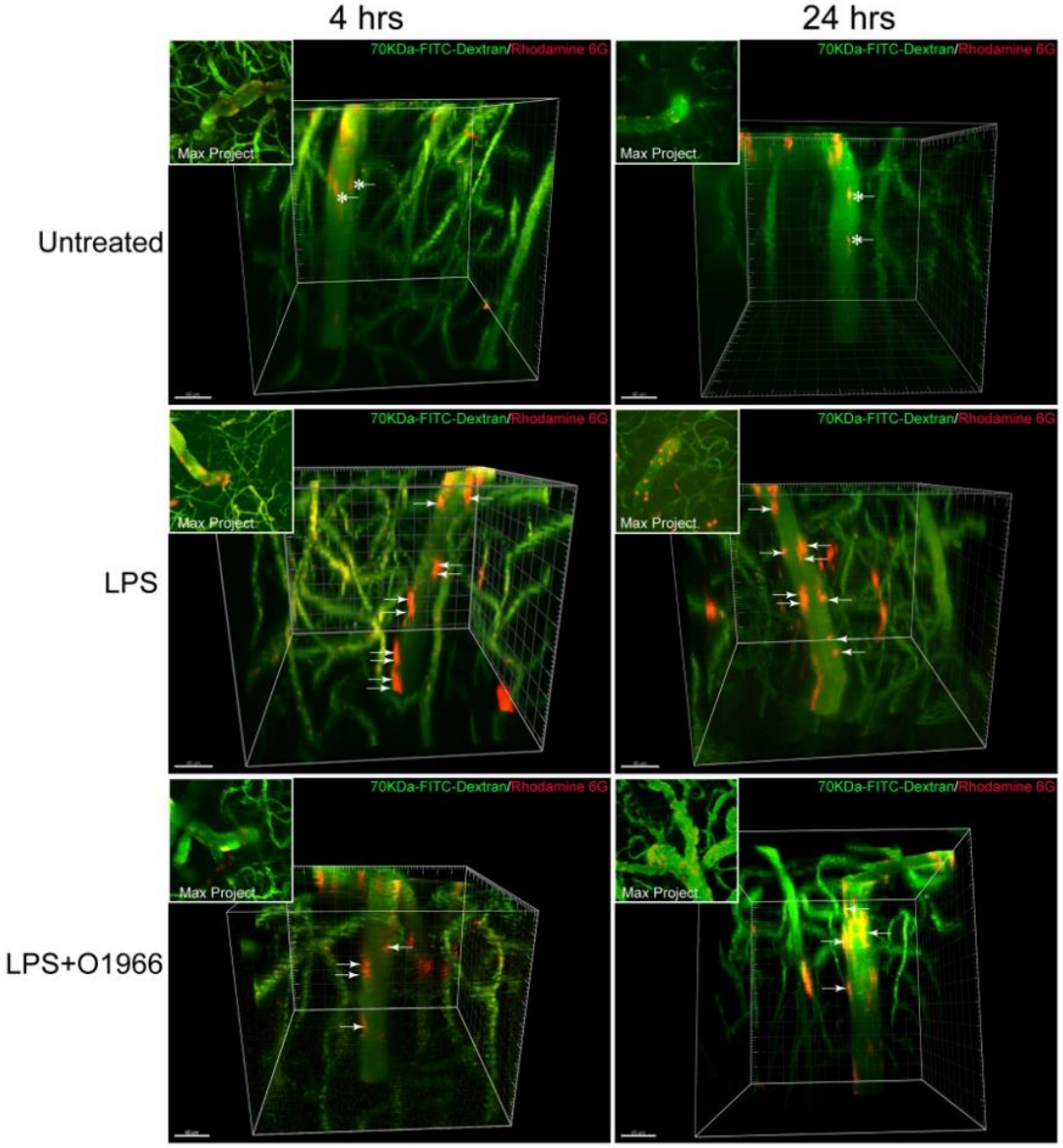
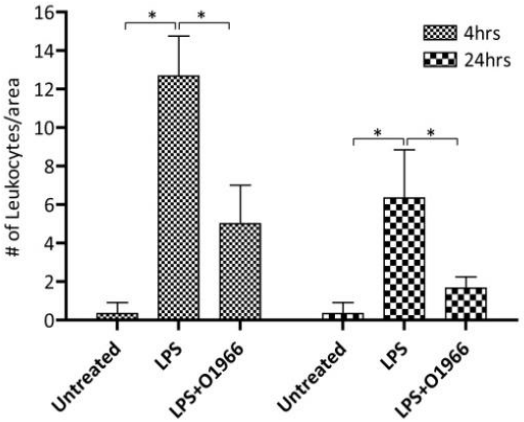
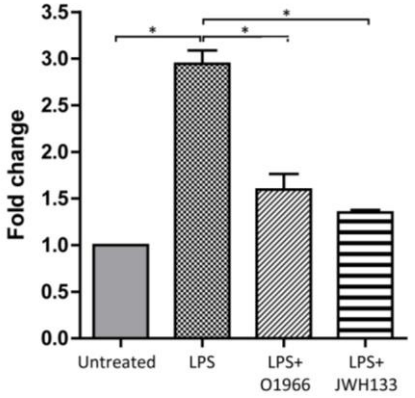
Ramirez et al. J. Neurosci 2012

CB_2 agonist inhibited leukocyte adhesion in post-capillary venules (multi-photon microscope imaging up to 800 microns) and diminished BBB permeability

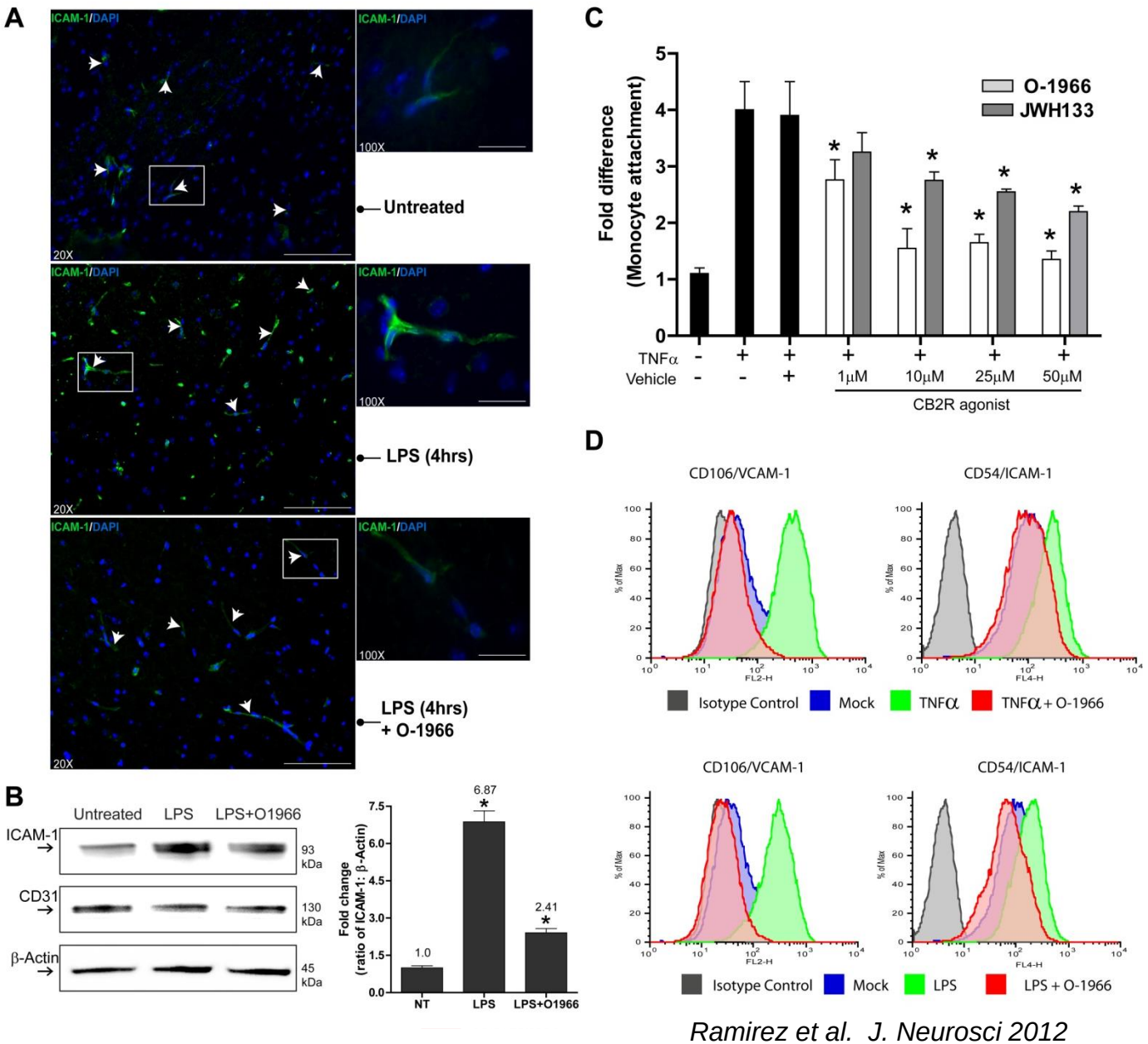






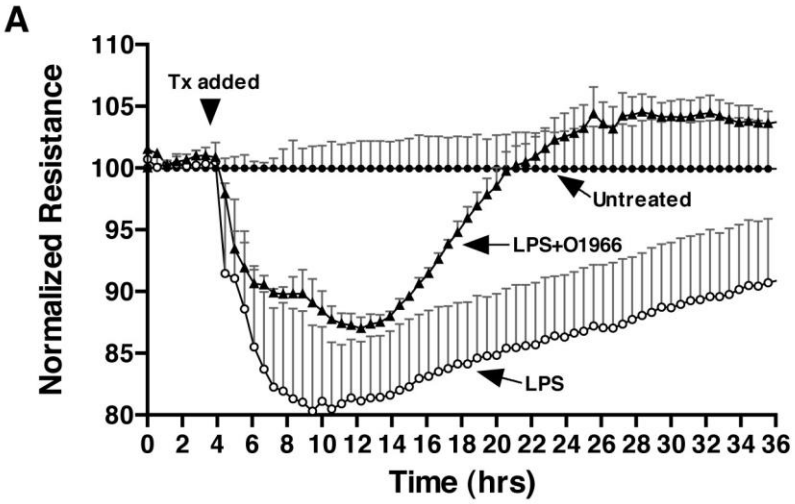
Ramirez et al. J. Neurosci 2012

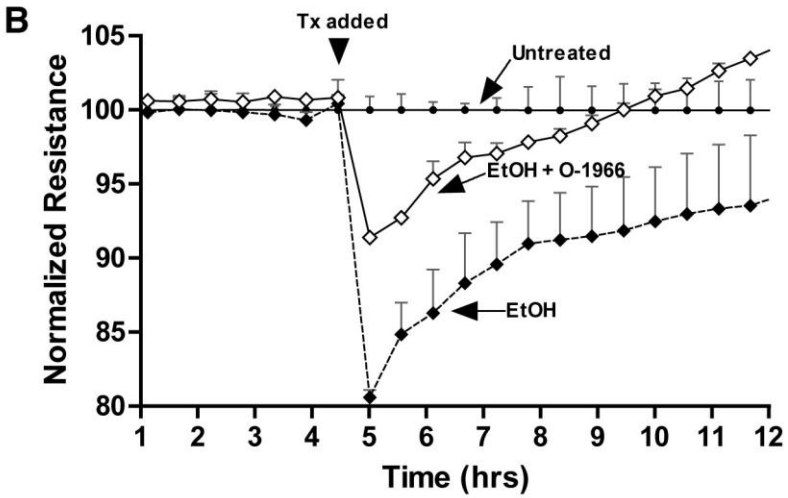
Inhibition of ICAM-1 and VCAM-1 by CB_2 agonist in activated brain endothelium *in vivo* and *in vitro*

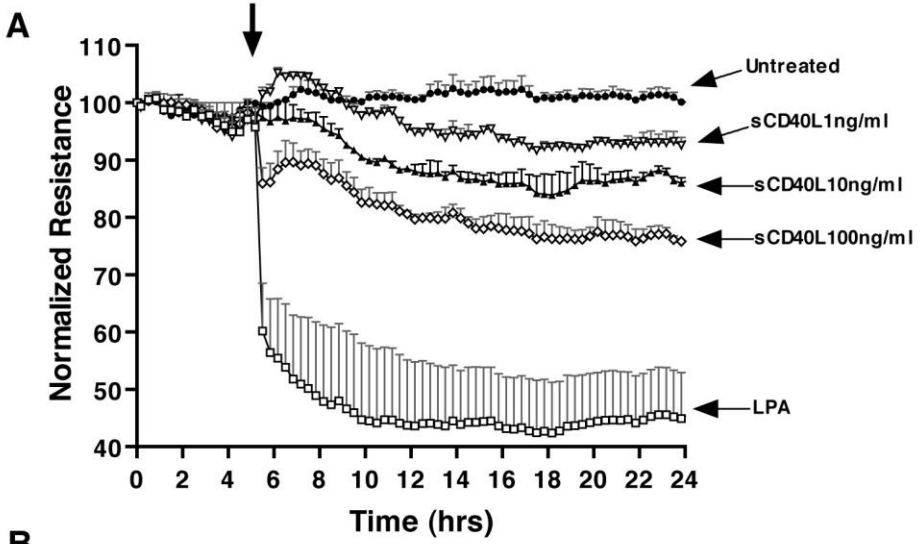


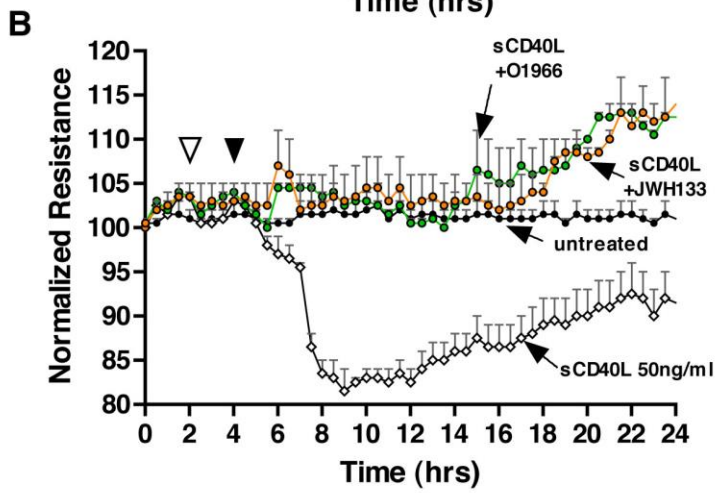
Ramirez et al. J. Neurosci 2012

CB_2 stimulation ameliorated barrier dysfunction after exposure to alcohol and inflammatory insults









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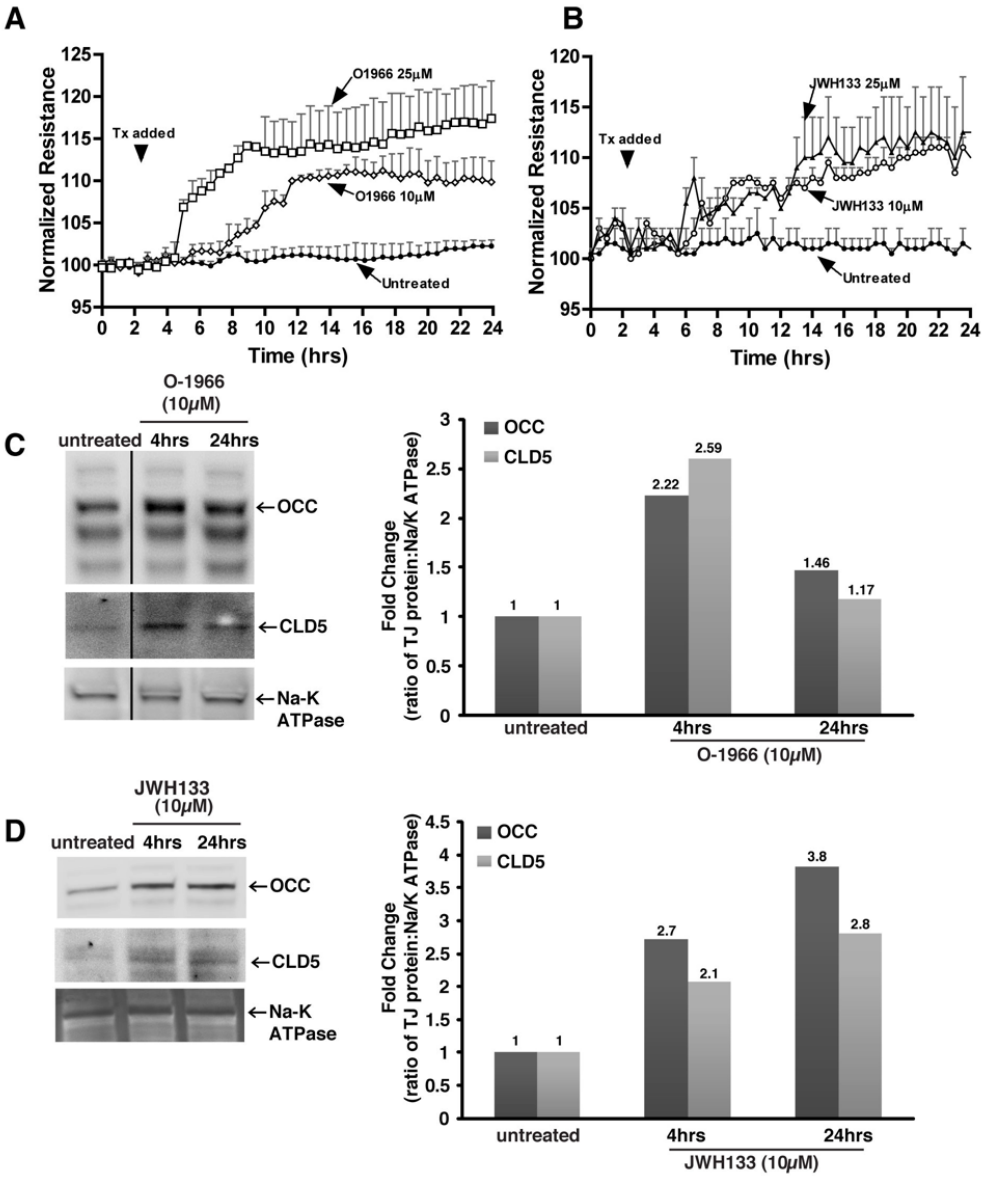
CB_2 stimulation increased barrier function



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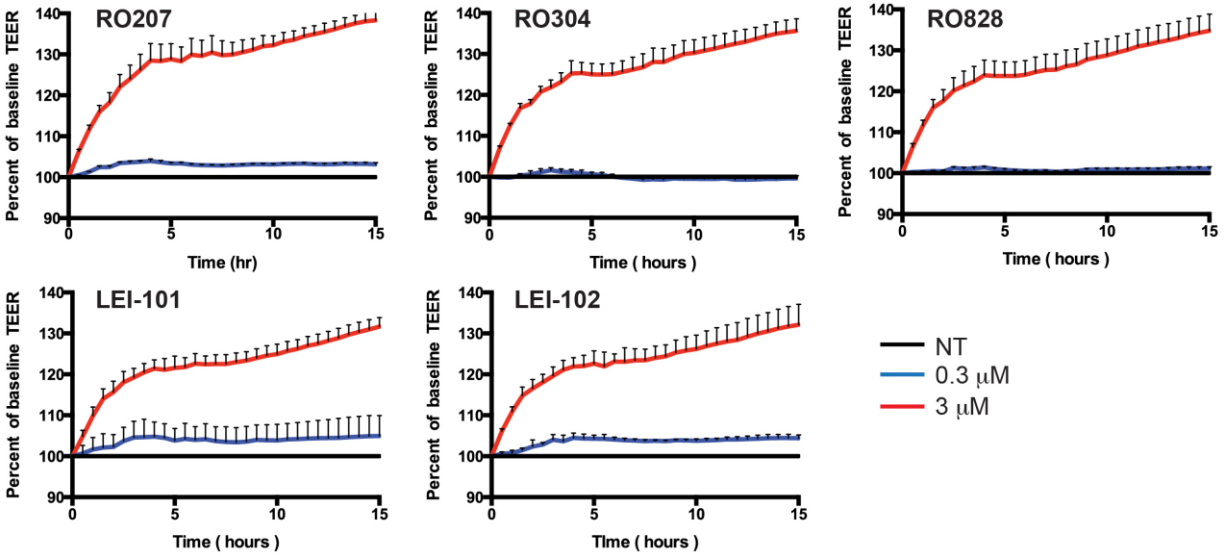
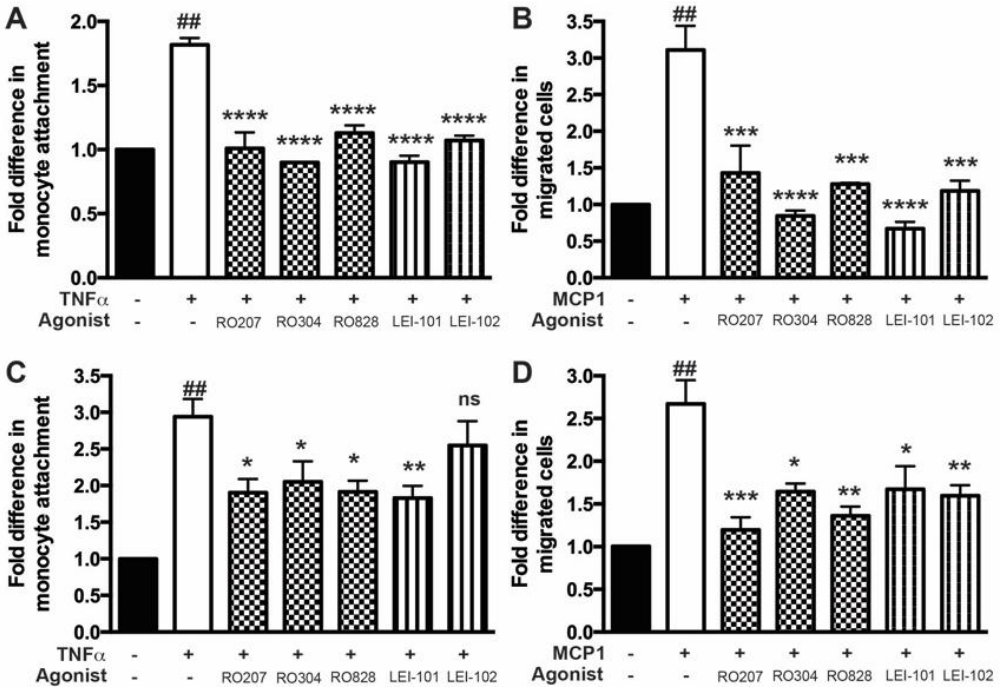
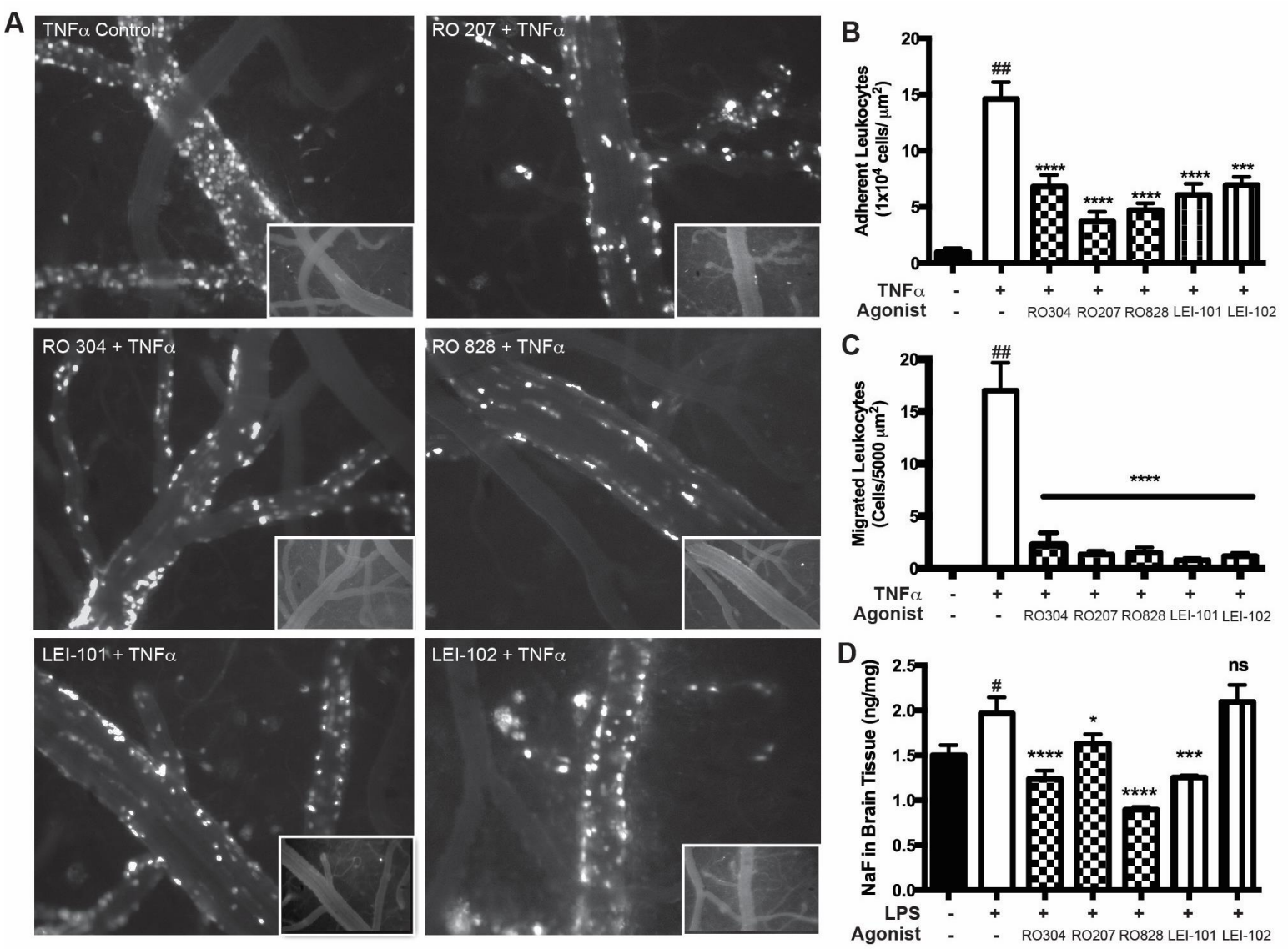
Novel selective, specific and orally bioavailable CB_2 agonists enhanced tightness of the barrier, diminished leukocyte adhesion/migration *in vitro* BBB models





Novel CB_2 agonists reduced leukocyte adhesion and migration in a model of aseptic encephalitis using a non-forceful feeding technique, and improve BBB tightness



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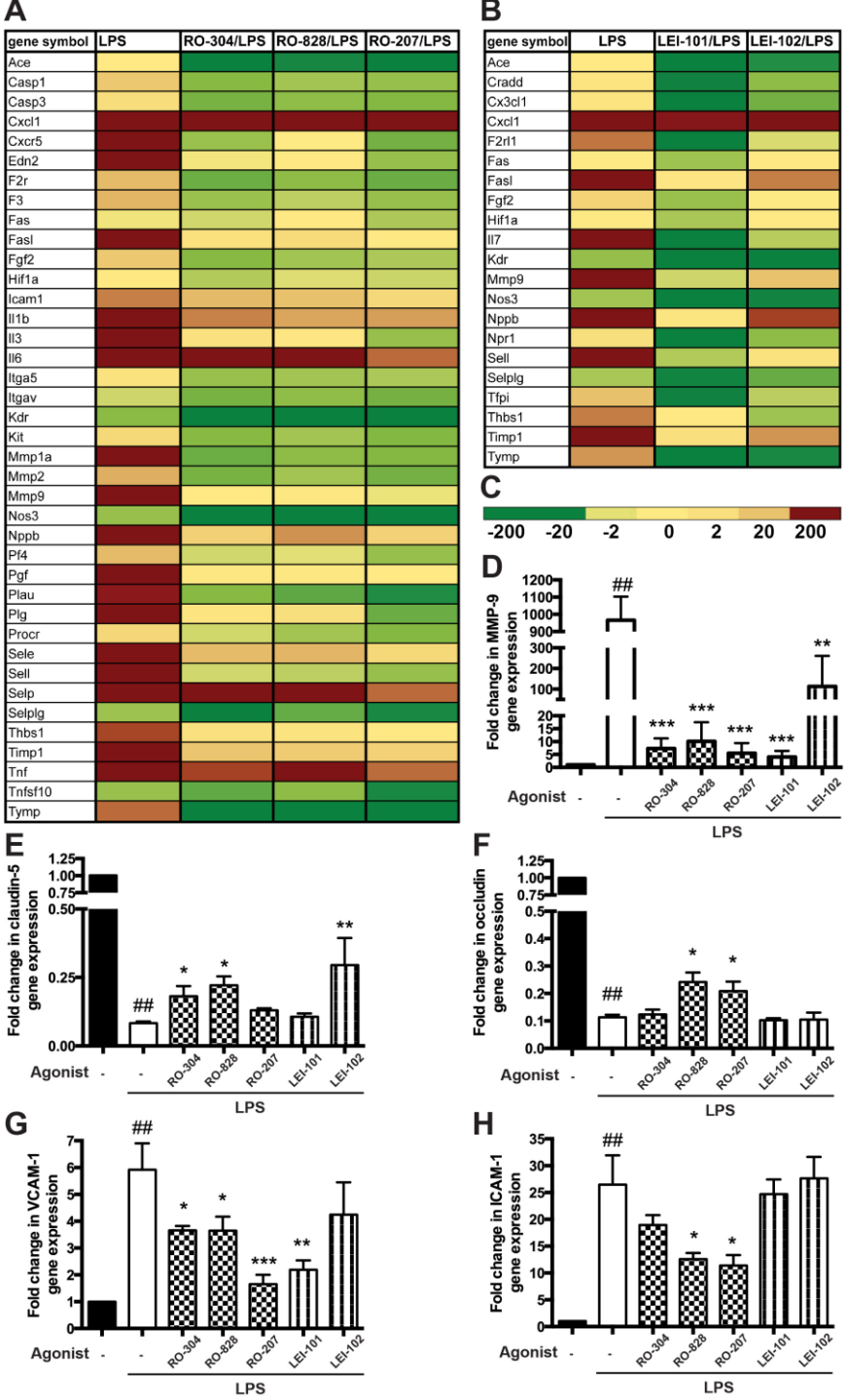
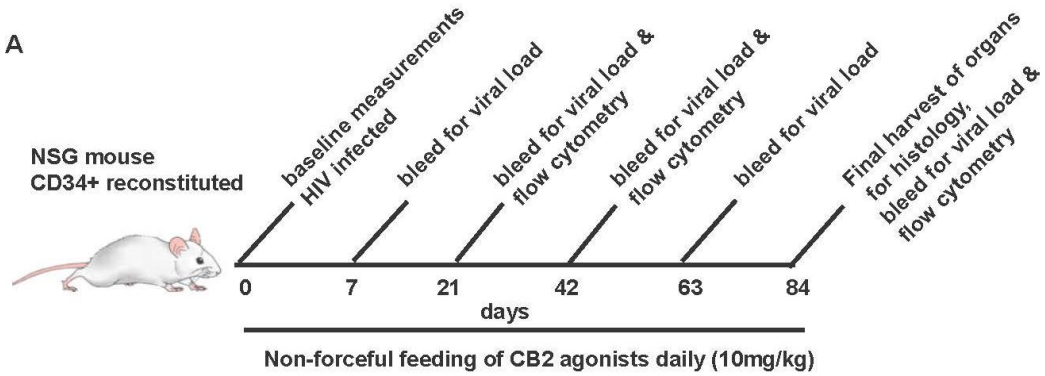
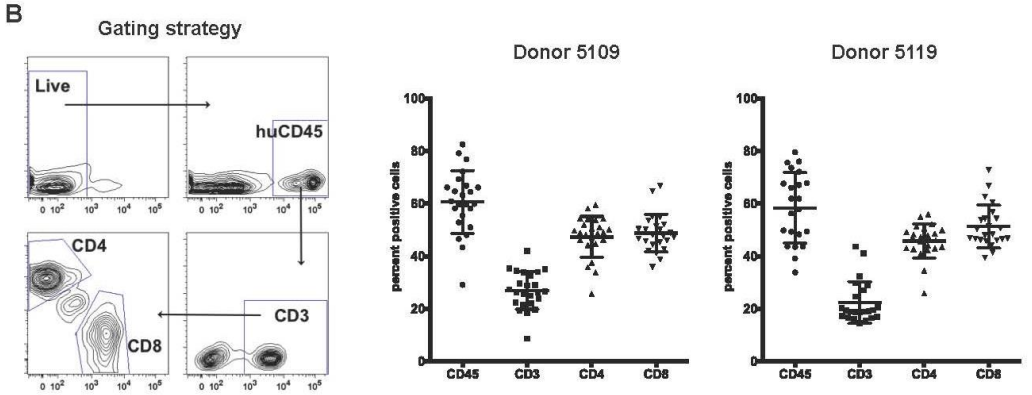
RO and LEI compounds diminished expression of inflammatory and barrier-compromising molecules in brain microvessels of mice with systemic inflammation (LPS injection)

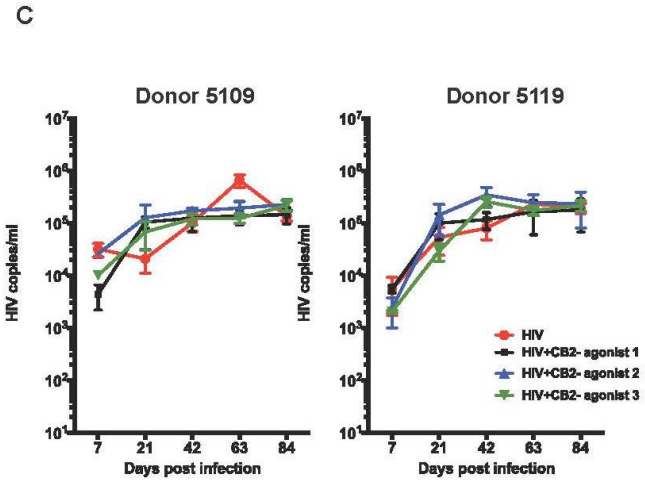
- RO-304, RO-828 and RO-207 compounds reversed these changes in 46, 18 and 32 genes, respectively,
- LEI-101 and LEI-102 attenuated alterations in gene expression in 33 and 4 genes respectively.

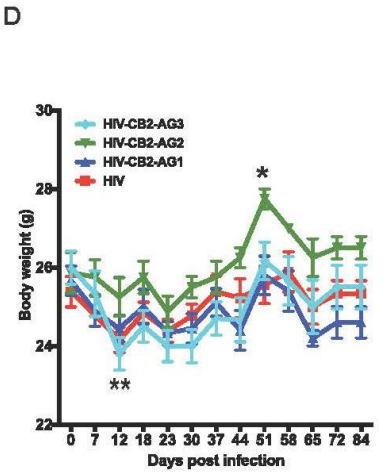


HIV-1 kinetics in huNSG-CB_2 agonist-treated mice



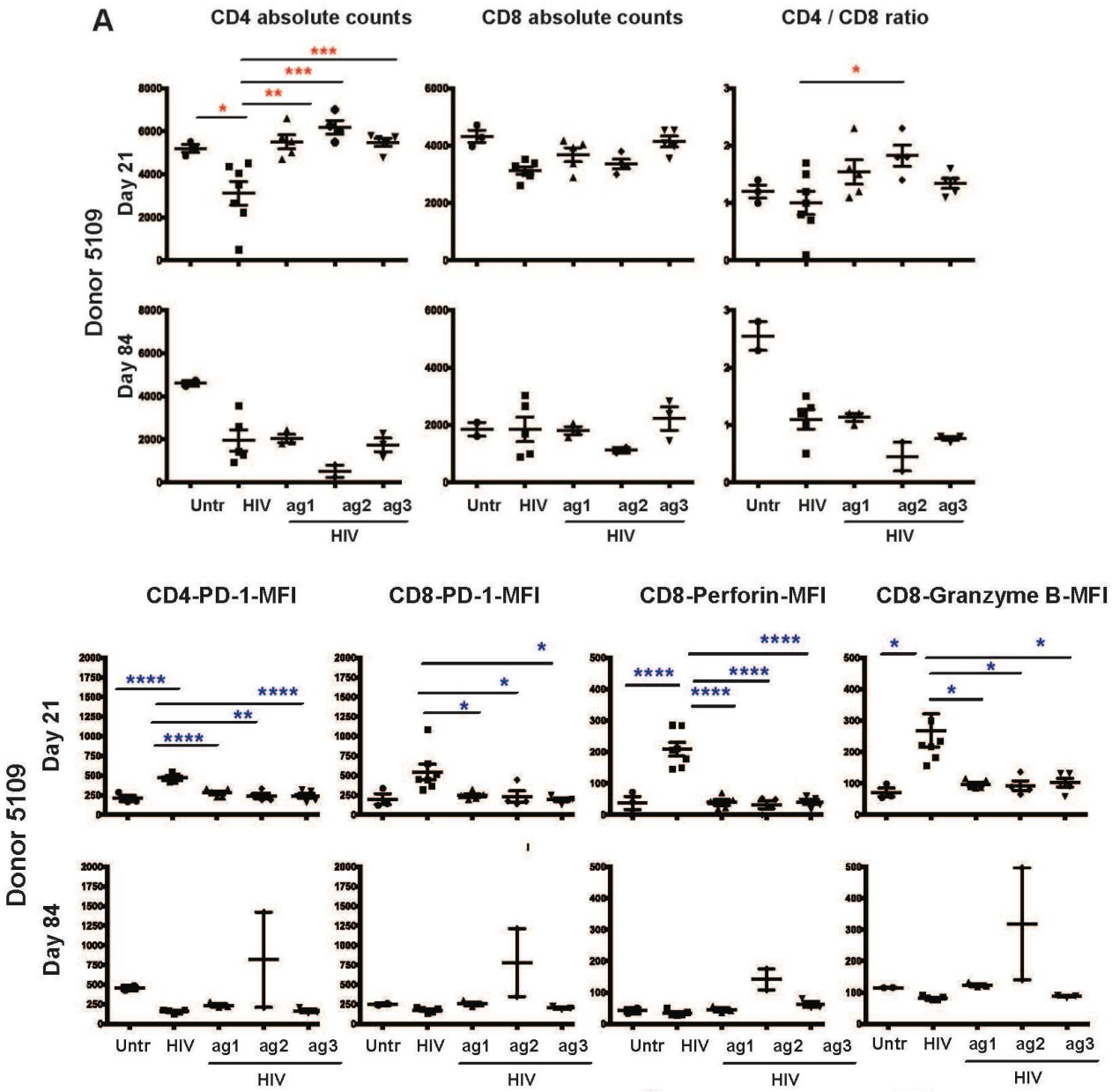




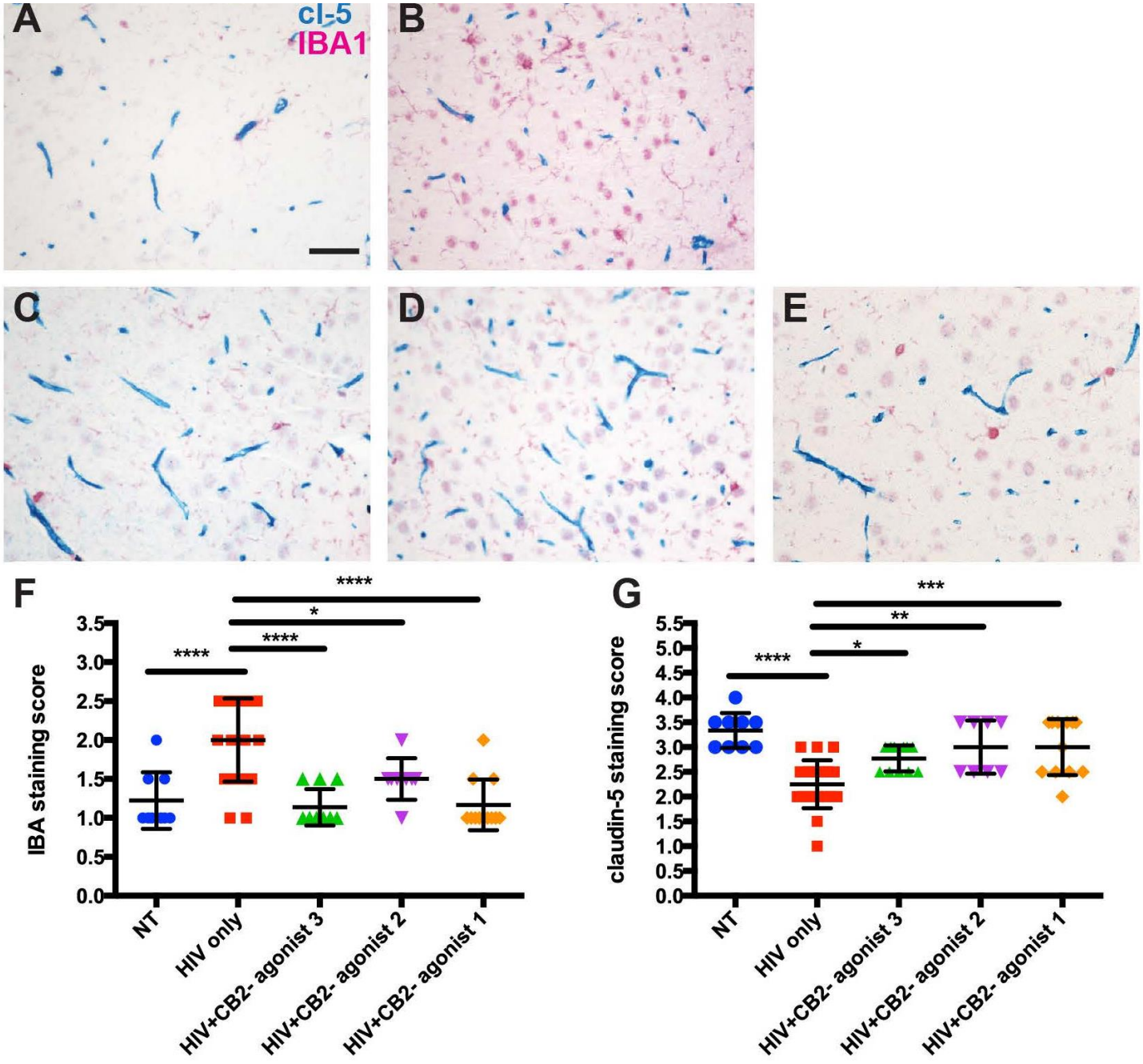


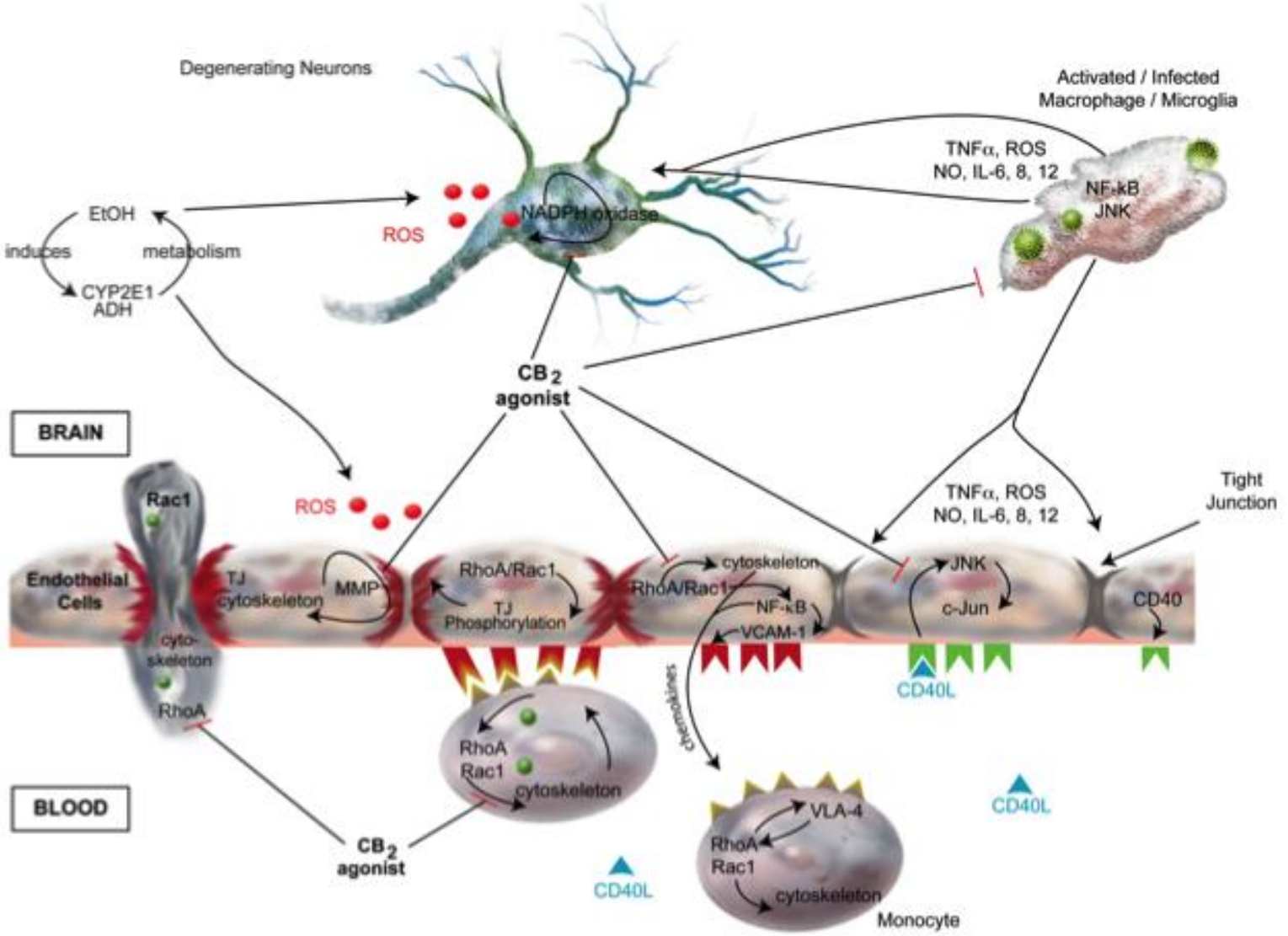
Blood immune profile after HIV infection and CB_2 agonist treatment

HIV increase of PD-1 in $CD4^+$ and $CD8^+$ cells and cytotoxic proteins, granzyme B and perforin in $CD8^+$ lymphocytes in HIV-infected animals, indicating substantial activation at week 3. Expression of these markers was suppressed by CB_2 agonists to levels seen in control, uninfected animals,



CB_2 agonists ameliorate neuroinflammation in chronic HIV infection in huNSG mice




Degenerating Neurons
Activated / Infected
Macrophage / Microglia
TNFα, ROS
NO, IL-6, 8, 12
NF-kB
JNK
EtOH
induces
metabolism
CYP2E1
ADH
ROS
NADPH oxidase
CB2
agonist
BRAIN
Rac1
ROS
TNFα, ROS
NO, IL-6, 8, 12
Tight
Junction
Endothelial
Cells
TJ
cytoskeleton
MMP
RhoA/Rac1
TJ
Phosphorylation
RhoA/Rac1
cytoskeleton
NF-kB
VCAM-1
JNK
c-Jun
CD40
cyto-
skeleton
RhoA
CD40L
RhoA
Rac1
cytoskeleton
Chemokines
BLOOD
CB2
agonist
CD40L
VLA-4
RhoA
Rac1
cytoskeleton
CD40L
Monocyte

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Complex effects drugs of abuse on peripheral and brain inflammatory and immune responses (tobacco smoke, e-cig, alcohol, methamphetamine, opioids) in the setting of HIV infection

Role of chronic neuroinflammation in BBB compromise in HIV infection and drugs of abuse

Anti-inflammatory interventions as potential treatment options (CB2 agonists, PPAR agonists and PARP inhibitors)