Cannabinoid Anti-inflammatory Properties: Implications for Treatment of HIV-Associated Neuroinflammation

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Disclosures

Serve on the Scientific Advisory Committee for GB Sciences Global Biopharma

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Outline

Introduction
- HIV and HAND
- cannabinoids
- cannabinoid receptors

Working Hypothesis

Results

Conclusions/Implications

Human Immunodeficiency Virus (HIV-1) Infection

- Global health concern
  - 37 million people infected worldwide
  - 1 million infected in the US
- Retrovirus that targets and infects cells of the immune system
  - $CD4^+$ T cells
  - Monocyte/macrophages
- Disease progression without treatment
  - Depletion in $CD4^+$ T cells
  - Chronic immune activation
  - Acquired immunodeficiency syndrome (AIDS)
- Antiretroviral therapy (ART) – controllable disease
  - Inhibits viral replication
  - Undetectable plasma viral load
  - Restoration in blood $CD4^+$ T cells
  - Increased life expectancy and higher quality of life

36.7 MILLION

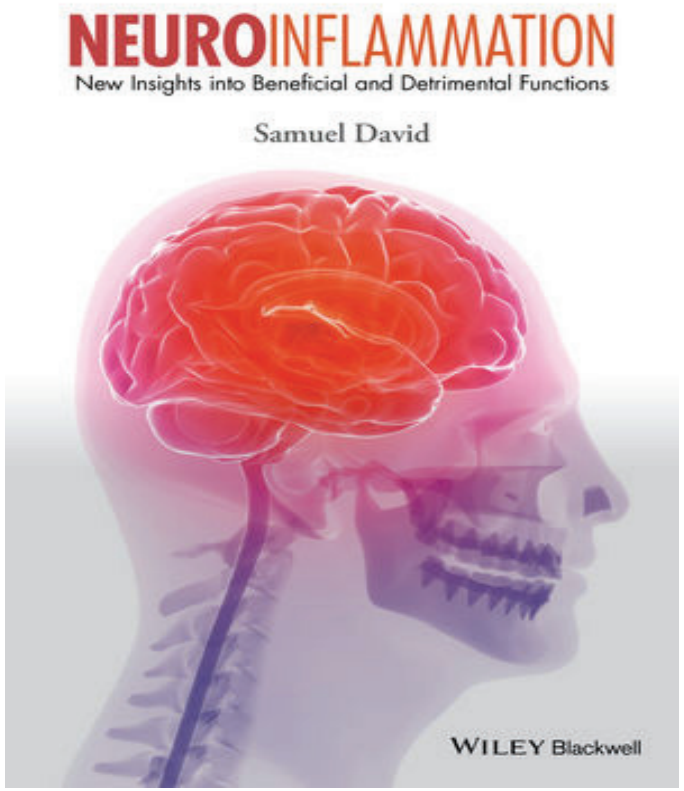
people worldwide are currently living with HIV/AIDS.

www.aids.gov

www.healthline.com

HIV-associated Neurocognitive Disorder (HAND)

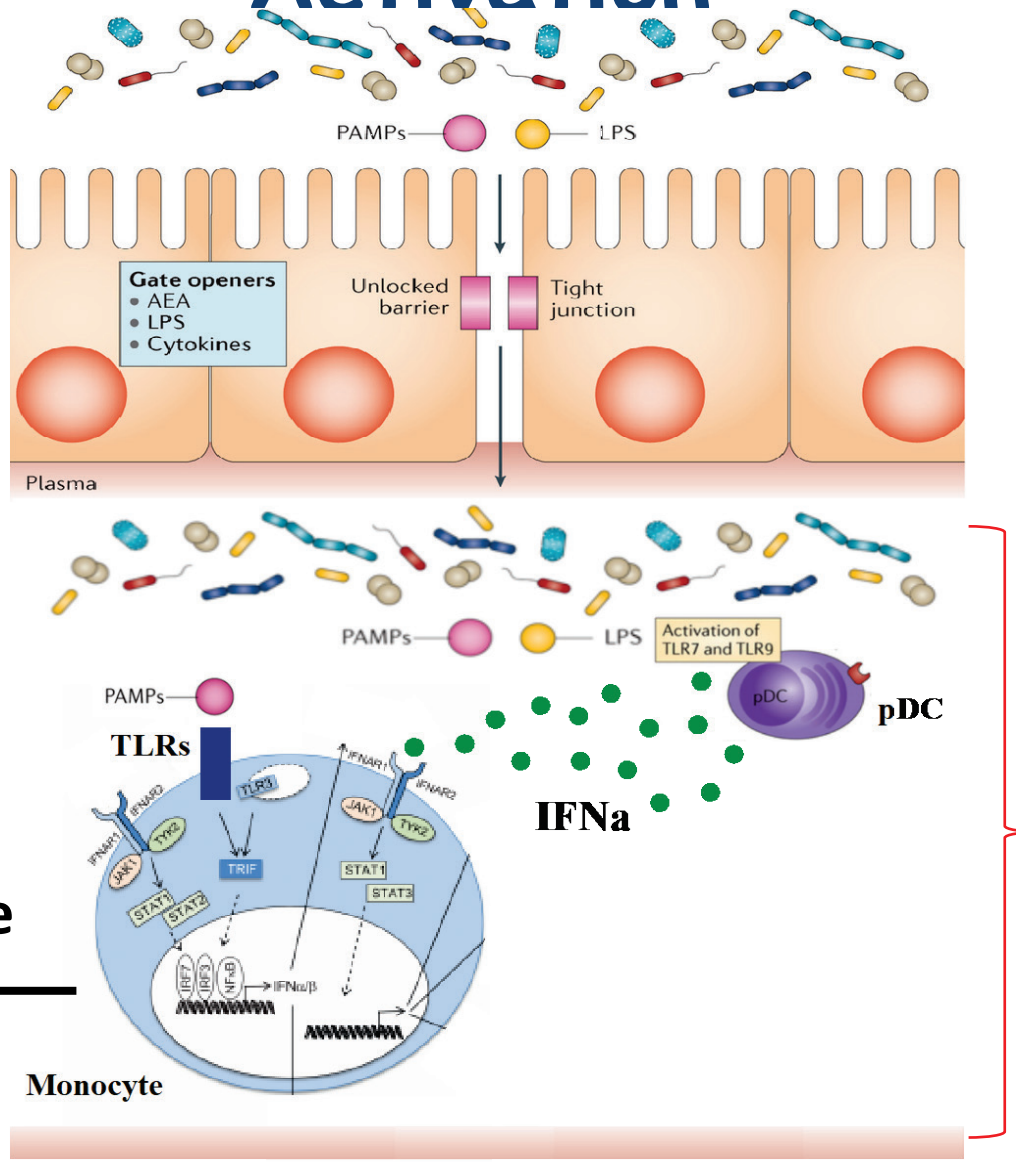
- **Prevalence:** 30-50%
  - Memory, self-control and complex thinking impacted
- **Cause:**
  - Dysfunction, damage and cell death of neurons
  - HIV does not directly infect neurons
  - **Major mechanism**: Low-level chronic neuroinflammation



David *et al.* 2015

Role of the Gut in Chronic Systemic Immune Activation

GUT

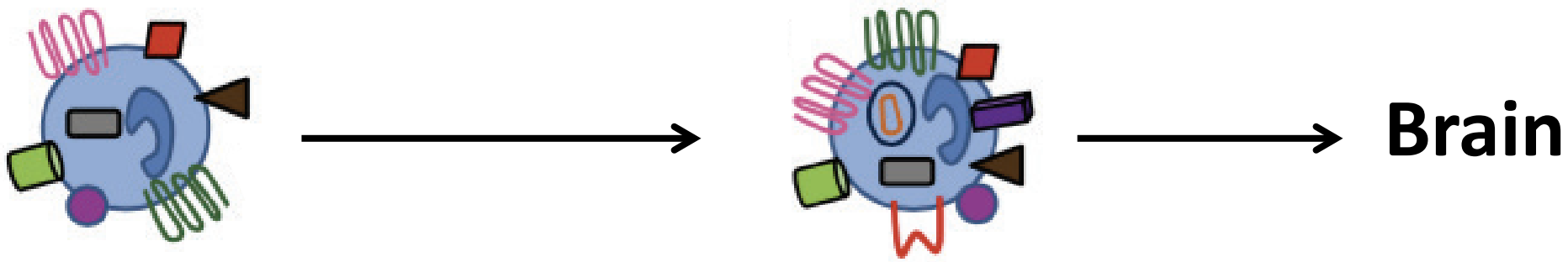


CD16+ monocyte activation

Circulating blood

Modified from Cani et al 2015, Ganguly et al 2013 and Cho et al 2014

CD16⁻ to CD16⁺ monocyte transition in the blood prior to entry into the CNS



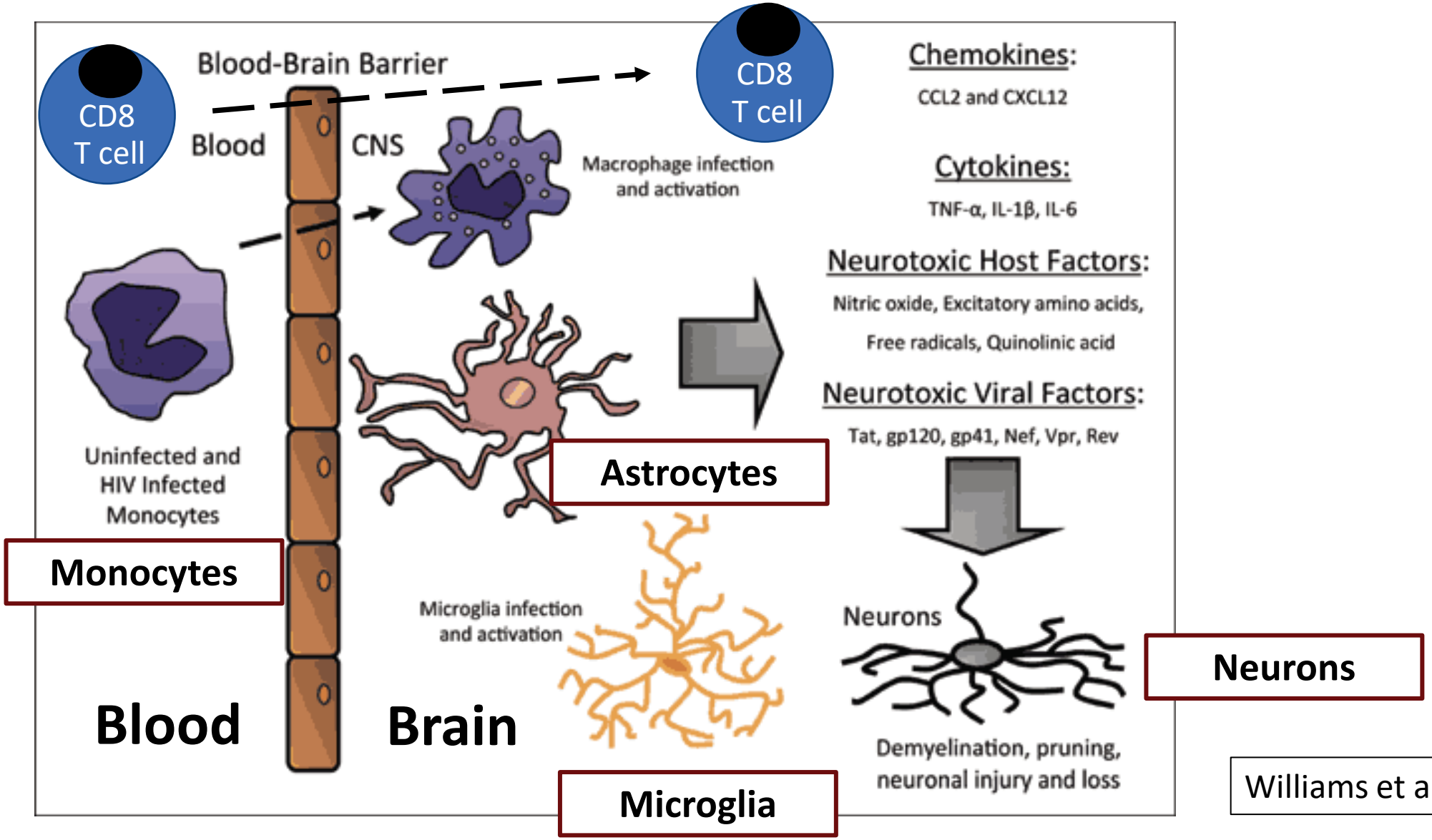
Classical ($CD14^+CD16^-$) monocyte

- Low susceptibility to HIV infection (low CCR5 expression)
- Minimal secretion of pro-inflammatory factors

Activated ($CD14^+CD16^+$) monocyte

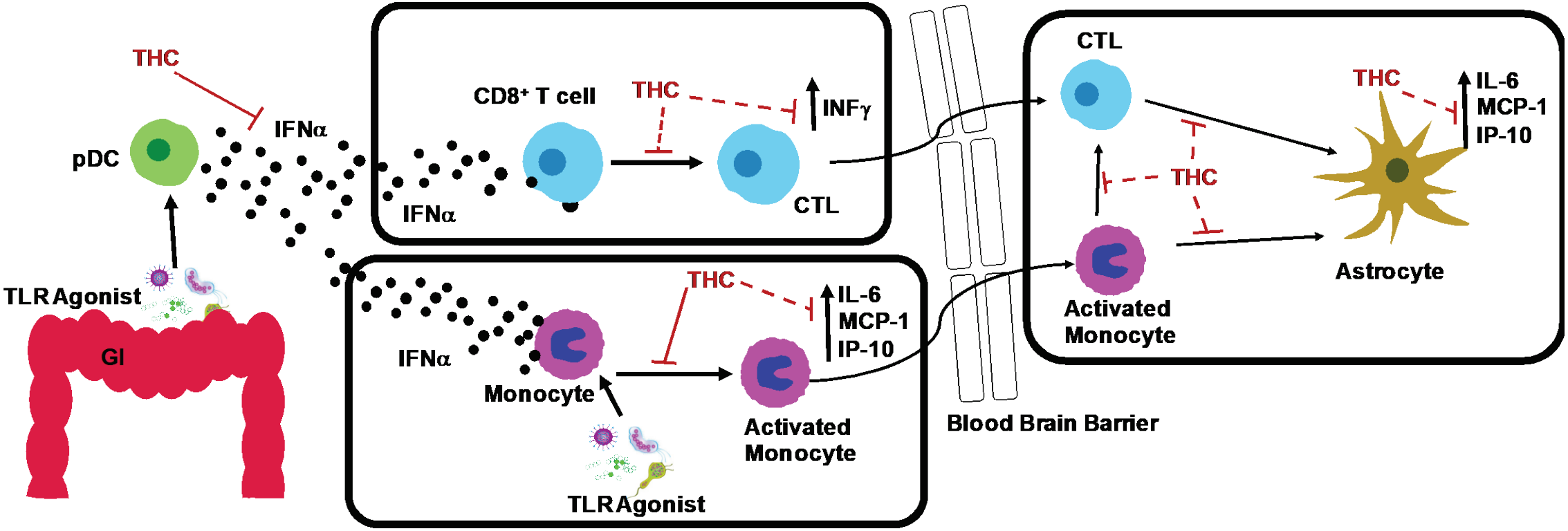
- High susceptibility to HIV infection (high CCR5)
- Serve as peripheral HIV reservoir
- Increased migratory capacity
- Transport HIV into brain
- Release pro-inflammatory factors

HIV-Associated Neuroinflammation



Williams et al 2014

Overview of Immune Activation Observed in HIV

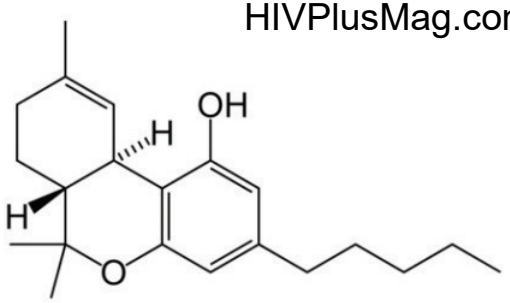


Cannabis Use by the HIV-Infected Population in the US is Prevalent

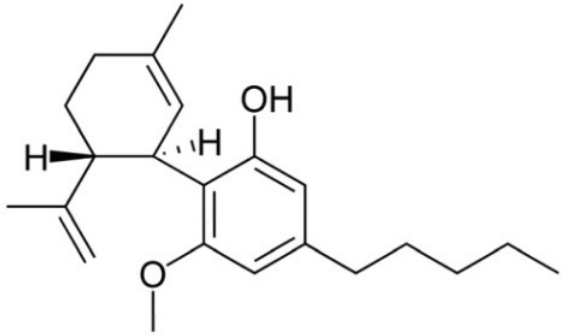
Cannabis Use by HIV-infected Individuals

- 23-56% of HIV patients use cannabis
- Relieve symptoms
  - Prevent nausea
  - Stimulate appetite
  - Relieve pain, anxiety and depression
- Cannabis contains 400 chemicals
  - >100 cannabinoids
  - **Δ^9-Tetrahydrocannabinol (THC) – 5-30%**
  - **Cannabidiol (CBD) – 0.2-1%**
- Smoking cannabis leads to rapid absorption of cannabinoids into circulation
- Peak plasma concentrations of THC range from **250-850nM** (within minutes of smoking)
- Rapidly distributed into tissues (e.g., brain)

HIVPlusMag.com

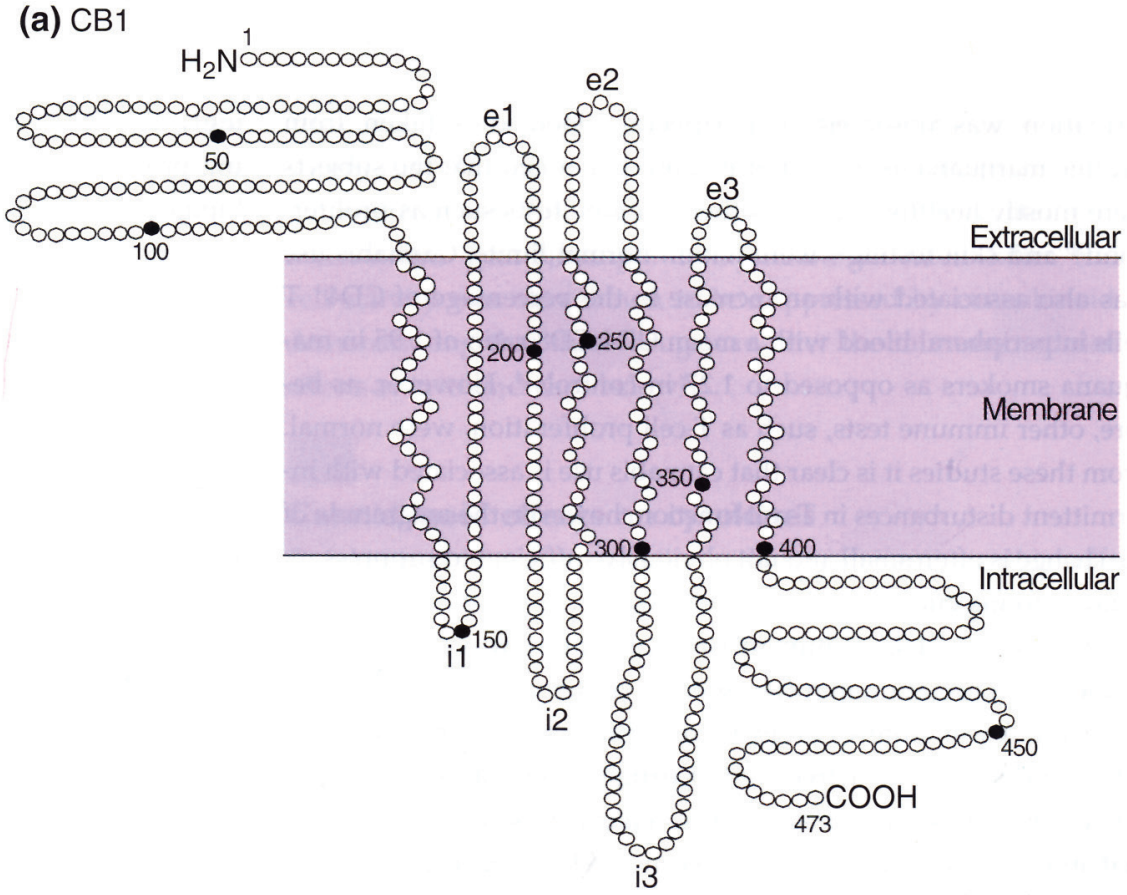


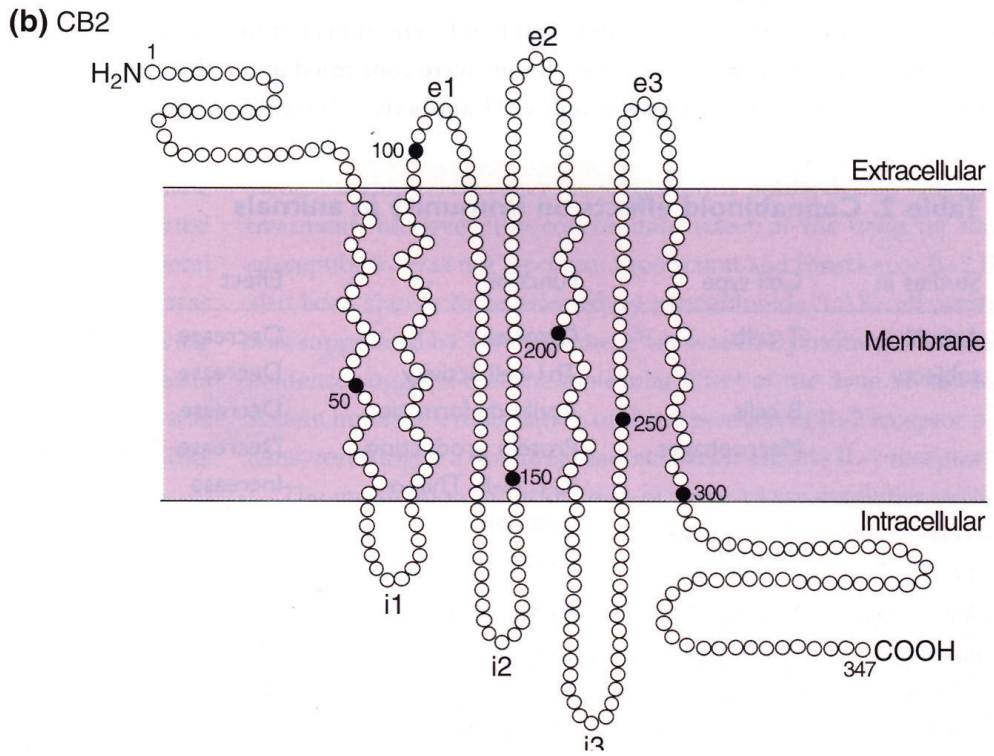
Δ^9-THC



CBD

Cannabinoid Receptors (CB1 and CB2)

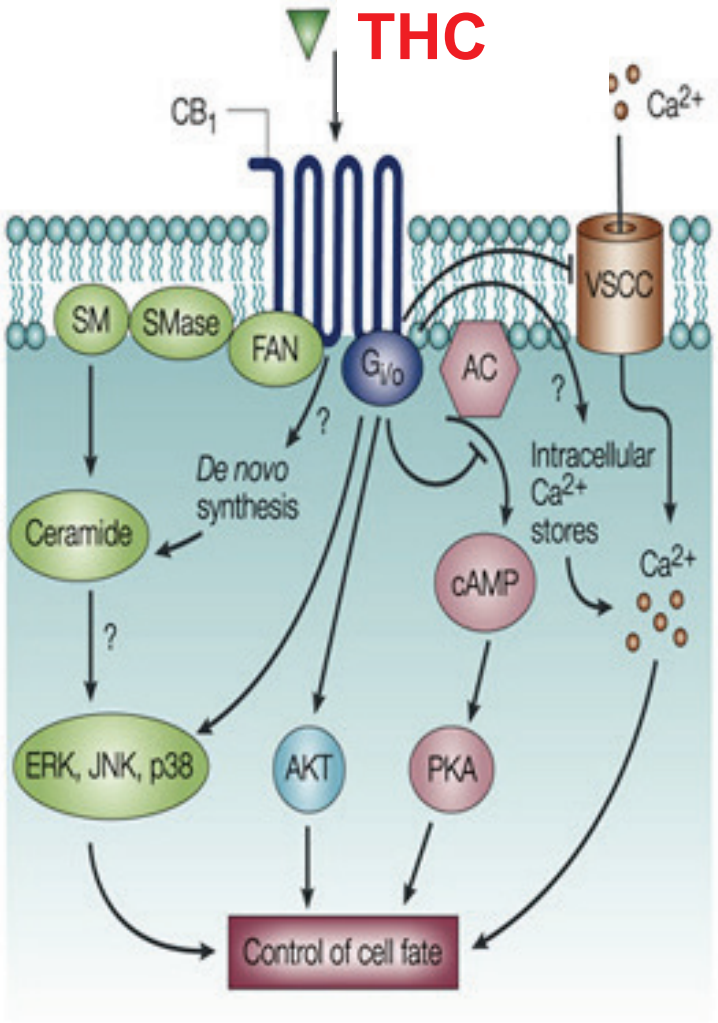




Klein, T.W., *et.al., Immunol. Today 1998*

Anti-inflammatory Effects of Cannabinoids

- Immune suppressive and **anti-inflammatory** properties
  - Pro-inflammatory cytokine production
  - Immune cell migration
  - T cell proliferation
  - Antibody response by B cells
- **THC:** cannabinoid receptors (CB1/CB2)
  - CB1 expression: CNS
  - CB2 expression: immune system
  - G-protein coupled receptors (GPCRs)
- **CBD**: independent of CB1/CB2
  - Mechanism unknown
- Human monocytes and pDC express CB1 and CB2



Guzman *et al.* 2003

Nature Reviews | Cancer

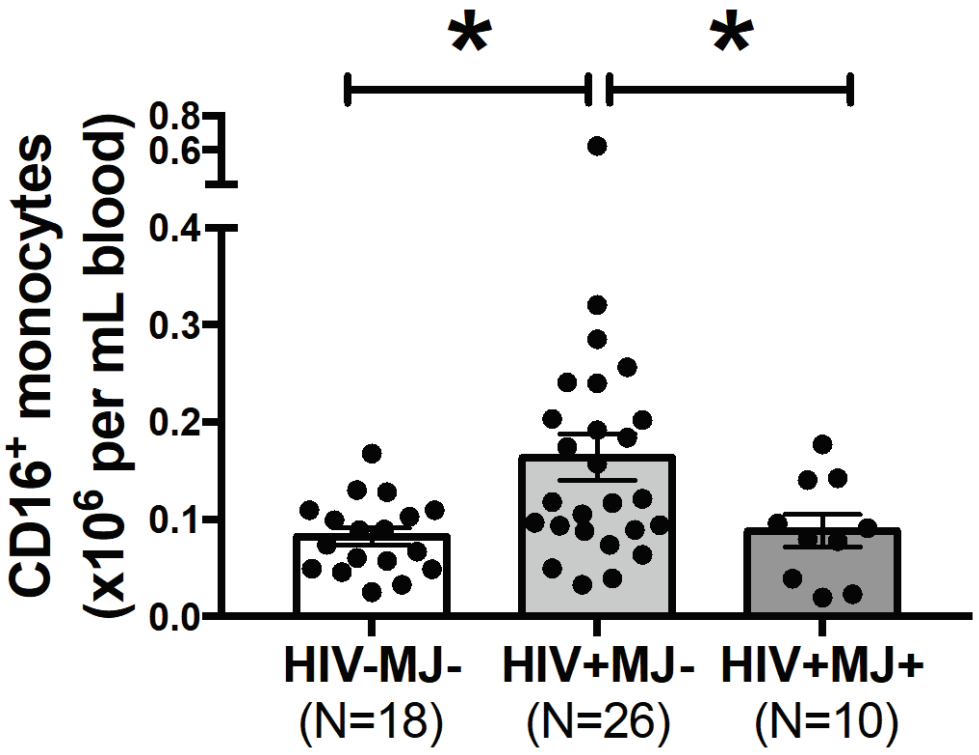
HIV-infected cannabis users have lower circulating $CD16^+$ monocytes and IFN-γ-inducible protein 10 levels compared with nonusing HIV patients

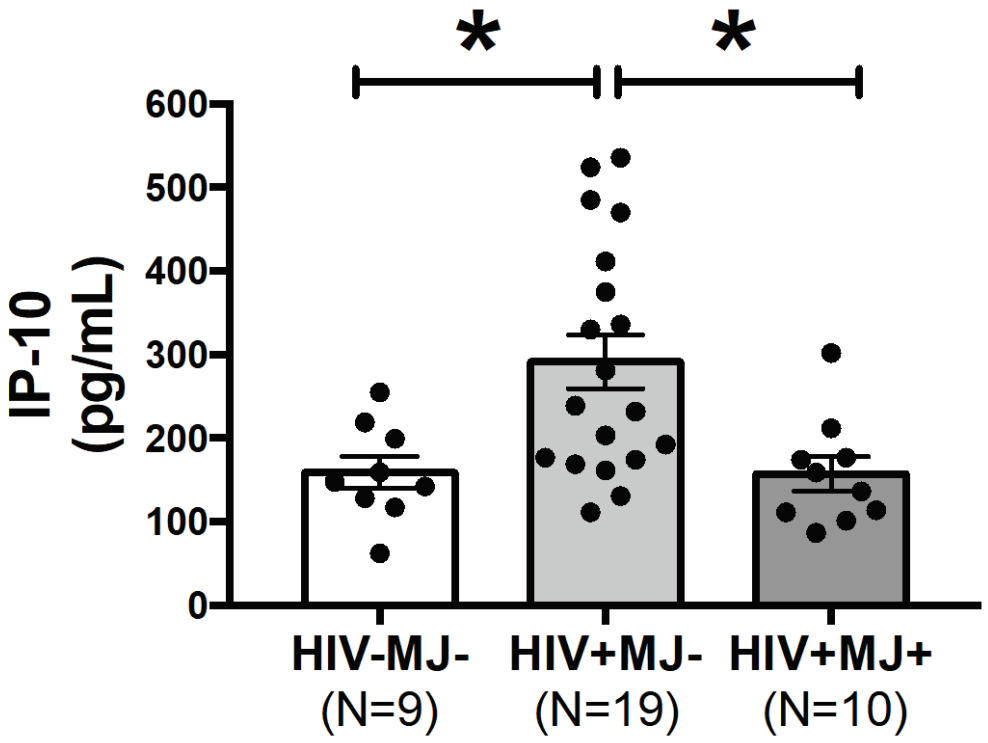
Michael D. Rizzo[a,b], Robert B. Crawford[b,c], Joseph E. Henriquez[b,c], Yasser A. Aldhamen[d], Peter Gulick[e], Andrea Amalfitano[d,e] and Norbert E. Kaminski[b,c]

AIDS 2018, **32**:419–429

Keywords: cannabis, $CD16^+$ monocyte, HIV, IFN-γ-inducible protein 10, Δ^9-tetrahydrocannabinol

Cannabis using HIV Patients Displayed a Lower Percentage of Activated Circulating (CD16⁺) Monocytes and Plasma IP-10 Compared to Non-using HIV Patients





MJ-: Non-cannabis users

MJ+: Cannabis users

AIDS 2018, 32:419–429

Working Hypothesis

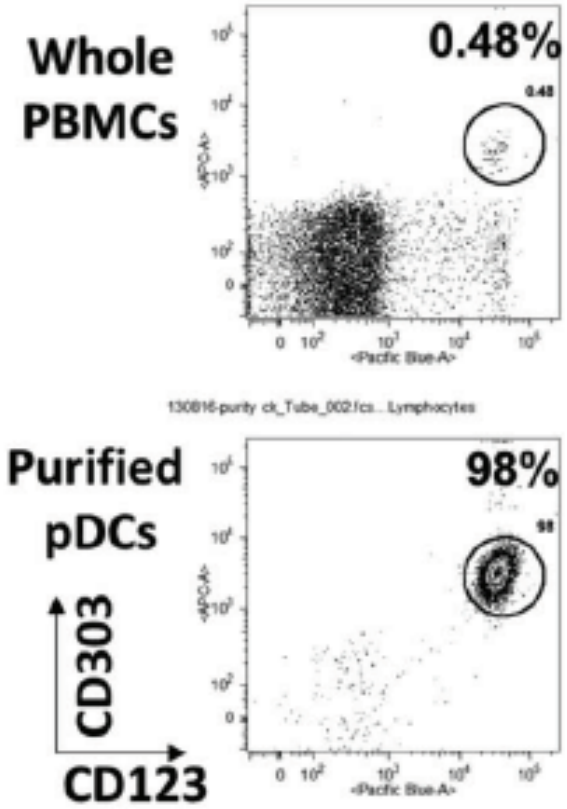
Cannabinoids suppress interferon-α-mediated monocyte activation in the periphery and their detrimental effects on astrocyte function, which are key processes in HIV-associated chronic neuroinflammation.

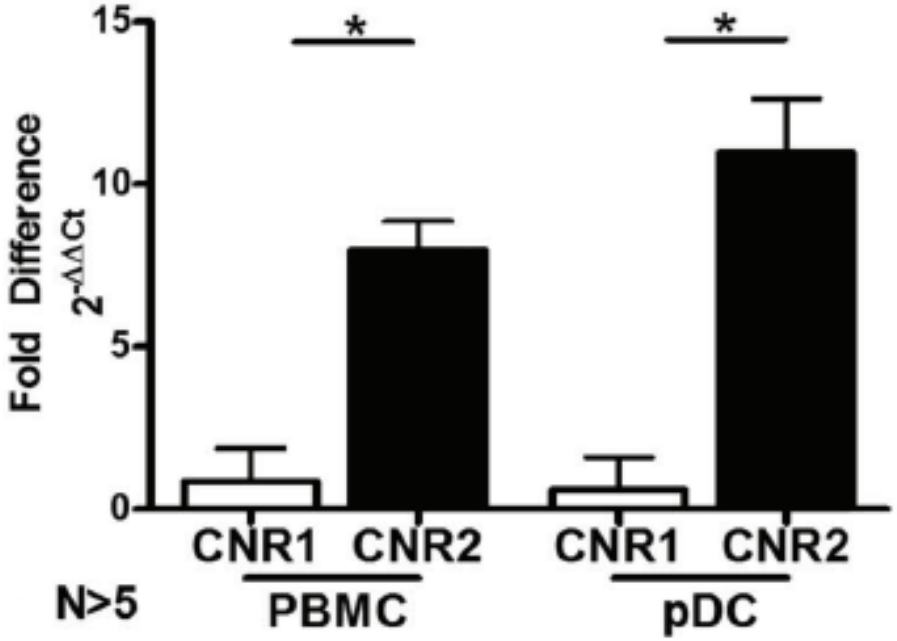
Results

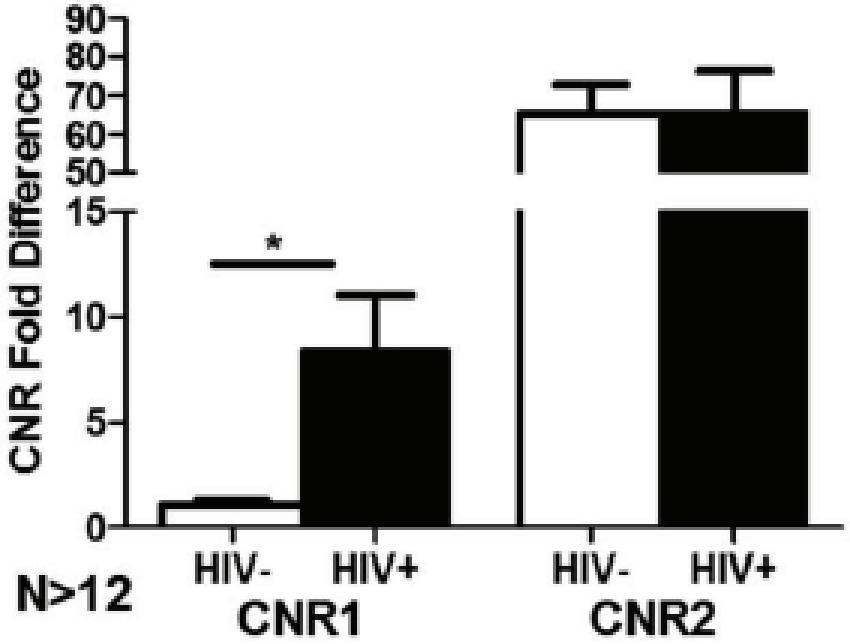
Effects of cannabinoids on peripheral inflammation in the context of HIV with a focus on **pDC - monocyte** interactions

Effects of cannabinoids on central inflammation in the context of HIV with a focus on **monocyte – astrocyte** interactions

Profile of CB1 and CB2 Expression by pDC in Healthy and HIV+ Subjects

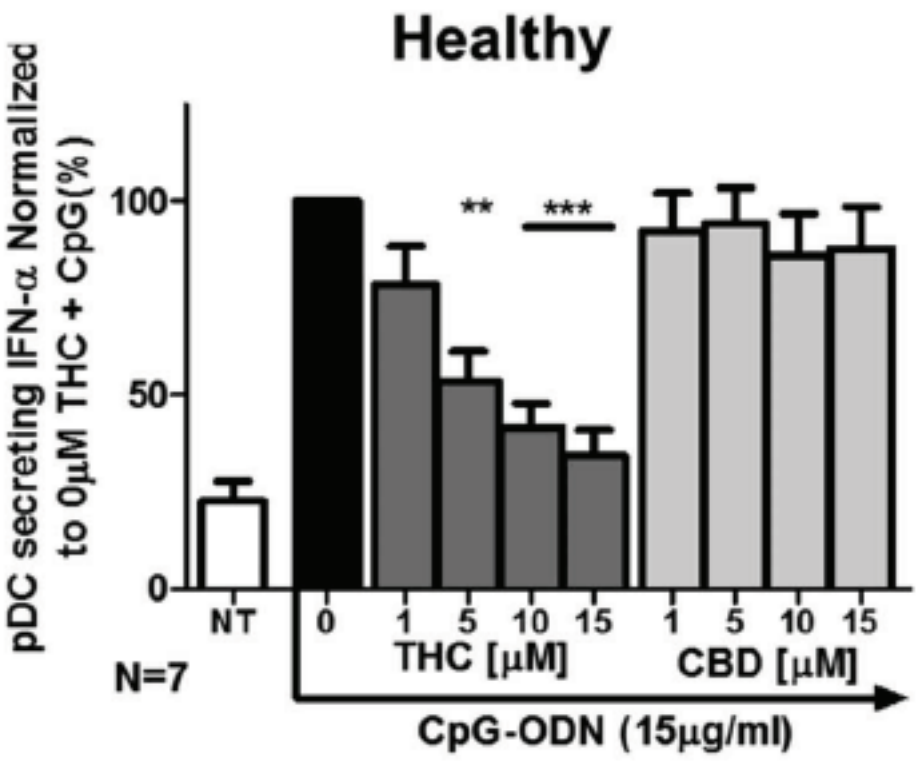


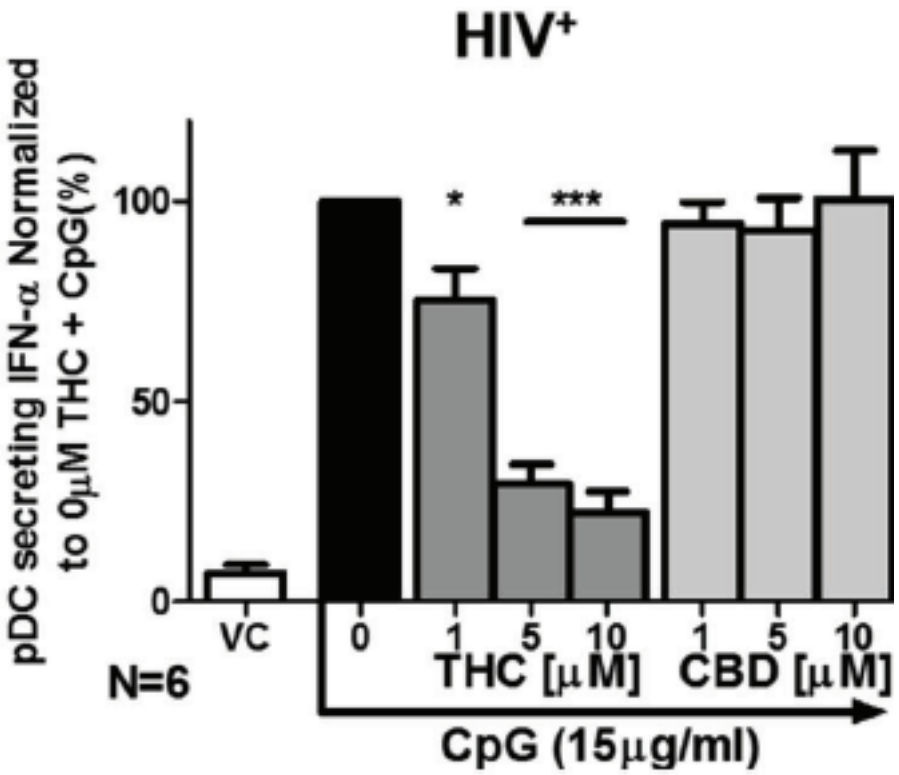


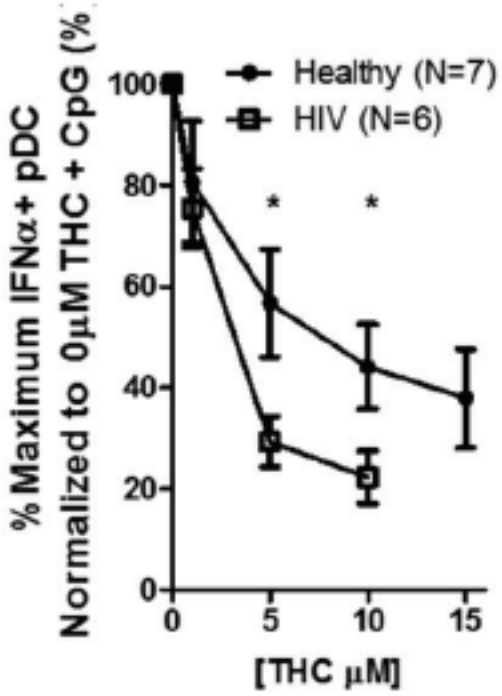


(J Acquir Immune Defic Syndr **2017;75:588–596)**

Suppression of pDC-derived IFNa in Healthy and HIV+ Subjects

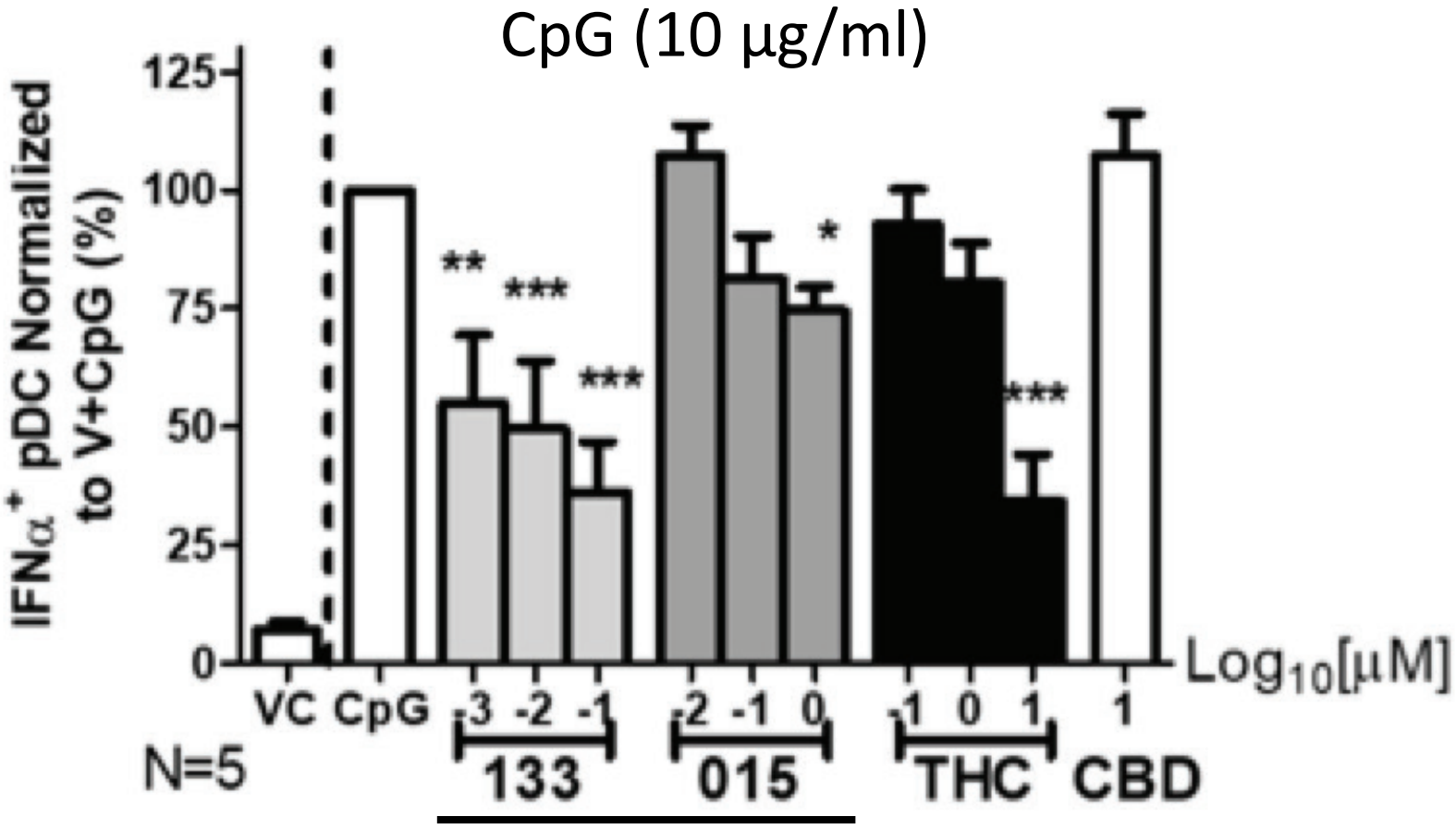






(J Acquir Immune Defic Syndr **2017;75:588–596)**

Suppress of IFNα⁺ pDC by CB2 Selective Agonist JWH-015 and JWH-133

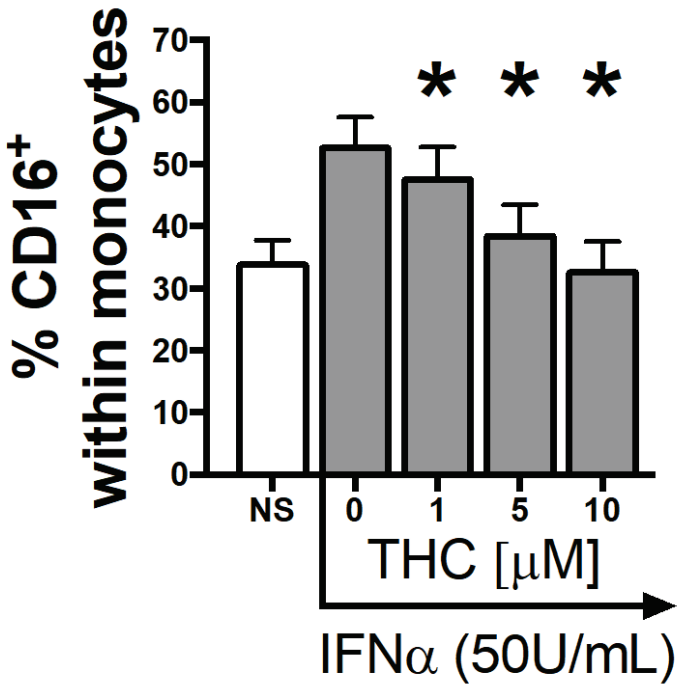


JWH Compounds (CB2 Selective)

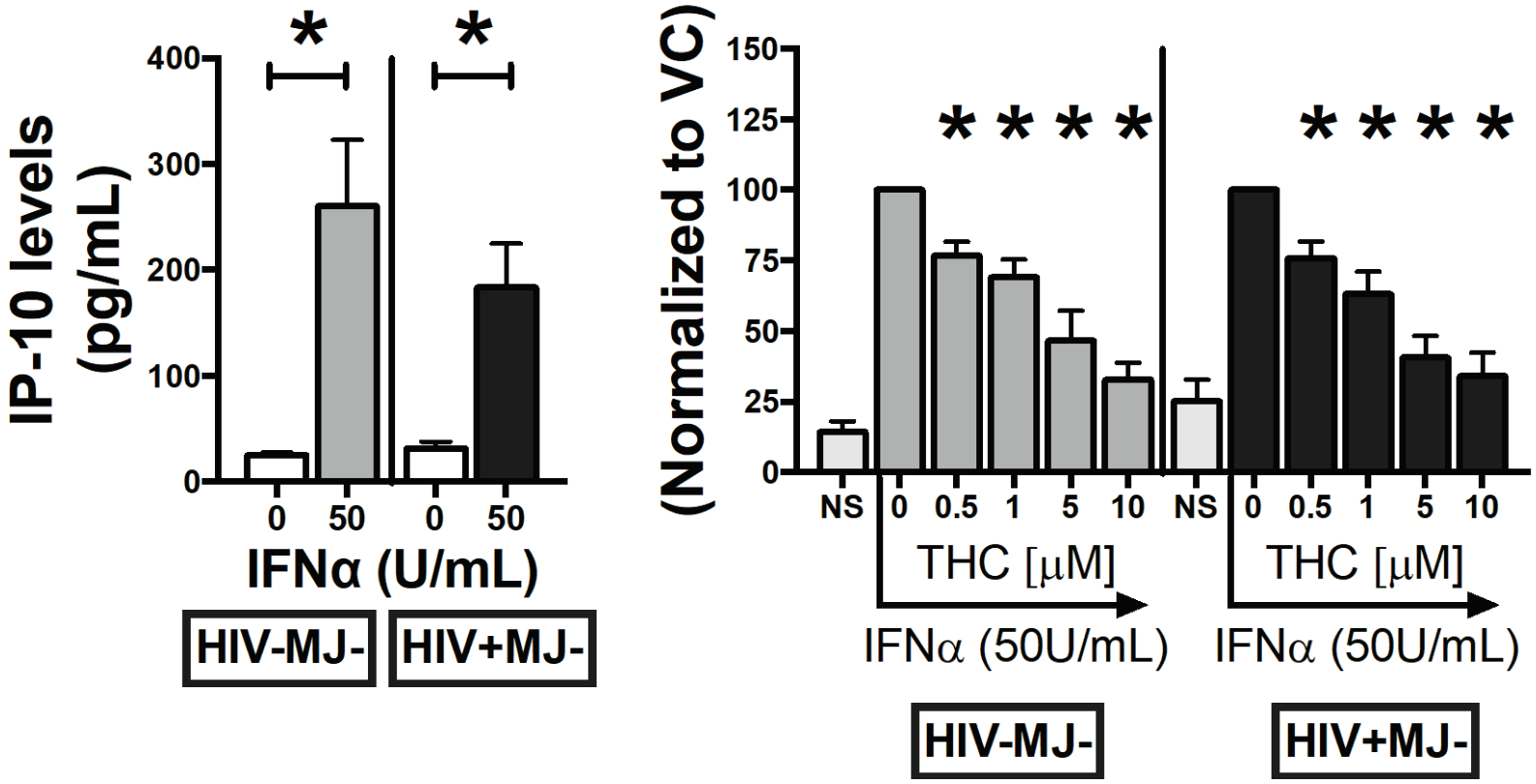
(Toxicol. Appl. Pharmacol. 369: 82-89, 2019)

In Vitro THC Treatment Suppressed CD16 Induction and IP-10 Production by Monocytes

HIV- PBMCs



Purified monocytes



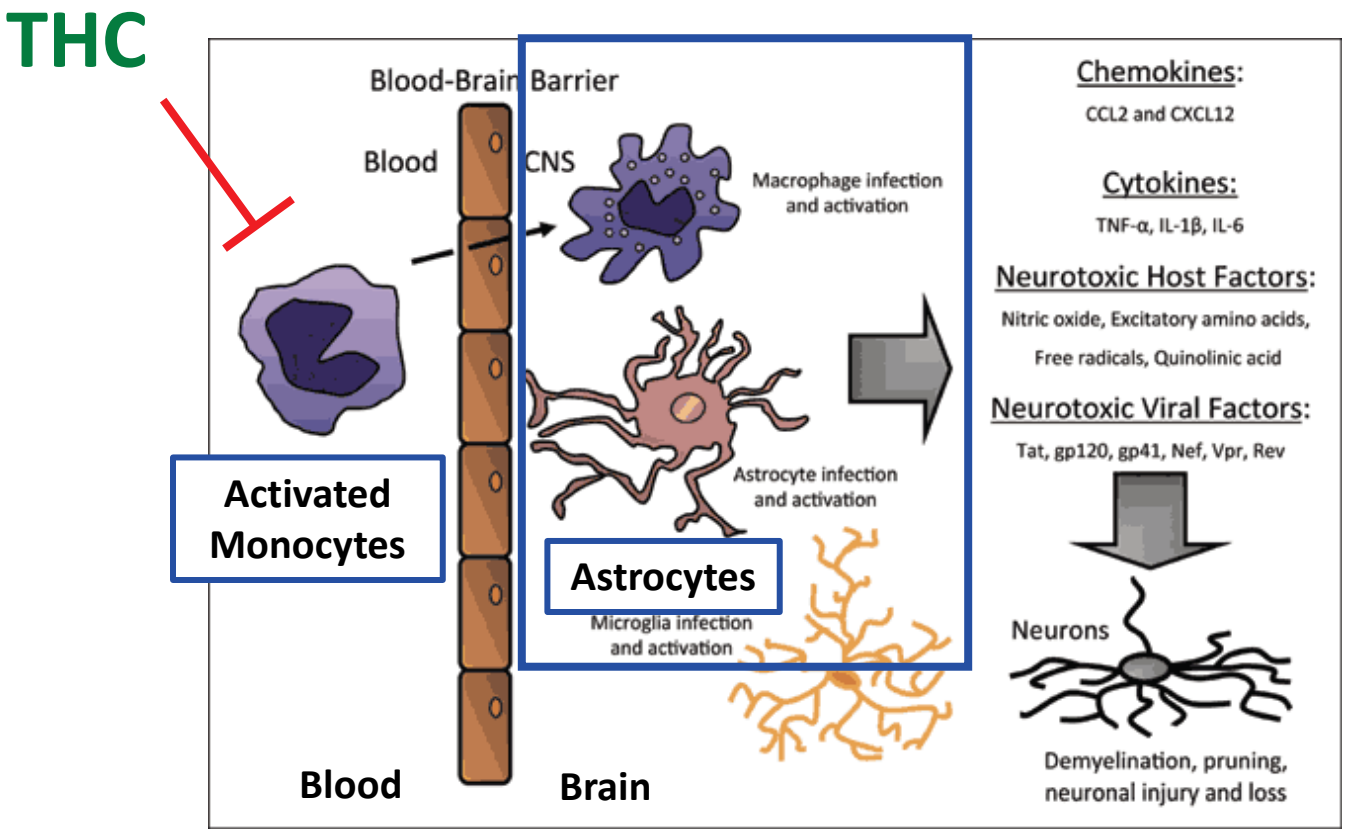
AIDS 2018, 32:419–429

Summary

- pDC express both CB1 and CB2 with CB2 being more highly expressed
- THC but not CBD treatment suppressed IFNα secretion by pDC with HIV+ subjects being more sensitive than HIV- to the THC-mediated suppression.
- CB2 selective agonists also suppressed IFNα secretion.
- THC suppressed IFNα–induced monocyte activation and IP-10 secretion.

Monocyte – Astrocyte Interactions

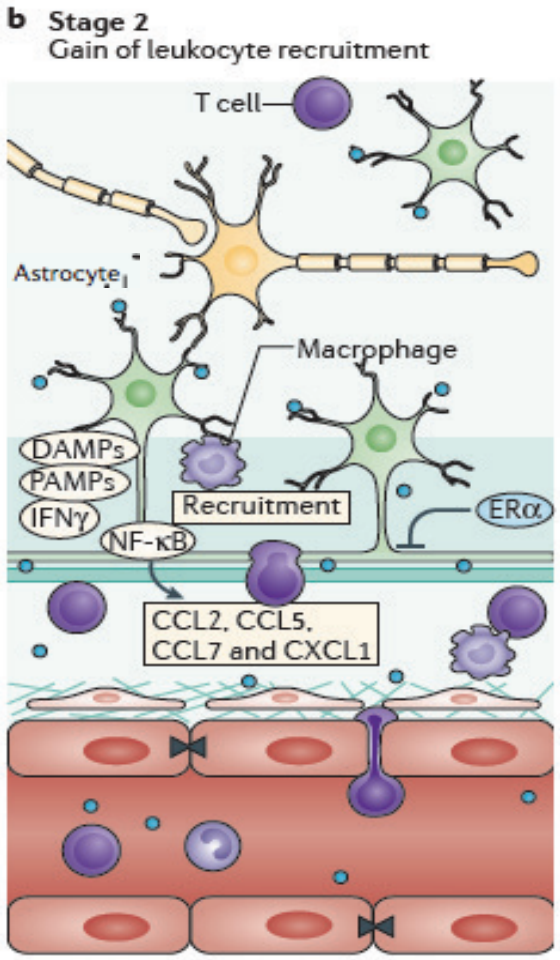
Determine if human monocytes, in combination with specific inflammatory factors, promote astrocyte inflammation



Williams *et al.* 2014

Chronic Astrocyte Inflammation in HAND

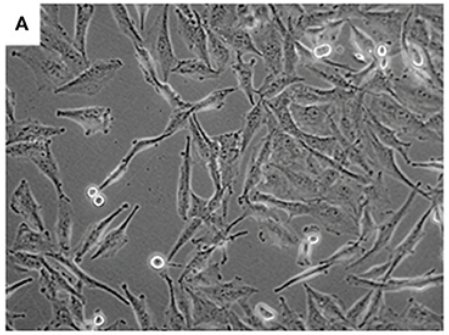
- Most abundant glial cell in the brain
- Numerous functions
  - Regulation of: BBB, blood flow, synaptic function and inflammation
- Chronically activated during HIV infection
- Stimulated by viral products and leukocyte-derived inflammatory factors
  - Close proximity to monocyte-derived macrophages
- **Secretion of cytokines and chemokines**
  - Increases BBB permeability (e.g. **IL-6**)
  - Promotes leukocyte recruitment
    - **MCP-1** (monocytes)/**IP-10** (T cells)
  - Positive feedback loop of inflammation



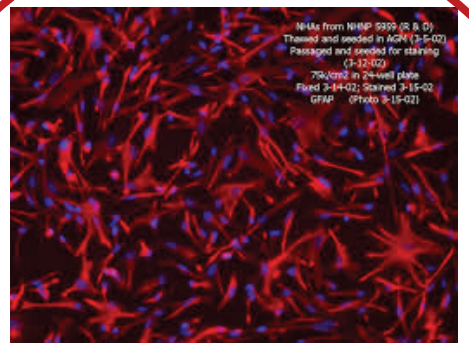
Sofroniew *et al.* 2015

Human Primary Monocyte-Astrocyte Co-Culture

Human blood
(HIV-negative)



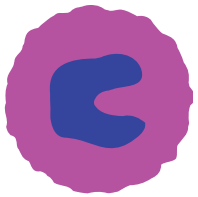
U251 astrocytoma line
(Sigma-Aldrich)

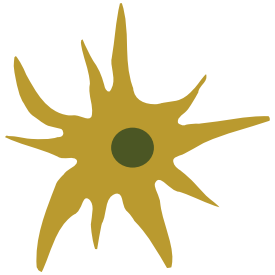


Primary fetal
astrocytes (Lonza) –
cerebral cortex

Magnetic-activated cell sorting (MACs)-Negative selection

Purified monocytes (>90%)





Astrocytes

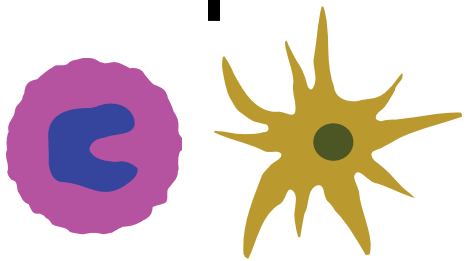
Picture (U251 cell line):
Katerji et al 2017

Human Monocyte-Astrocyte Co-Culture: Experimental Design

Monocytes cultured with astrocytes

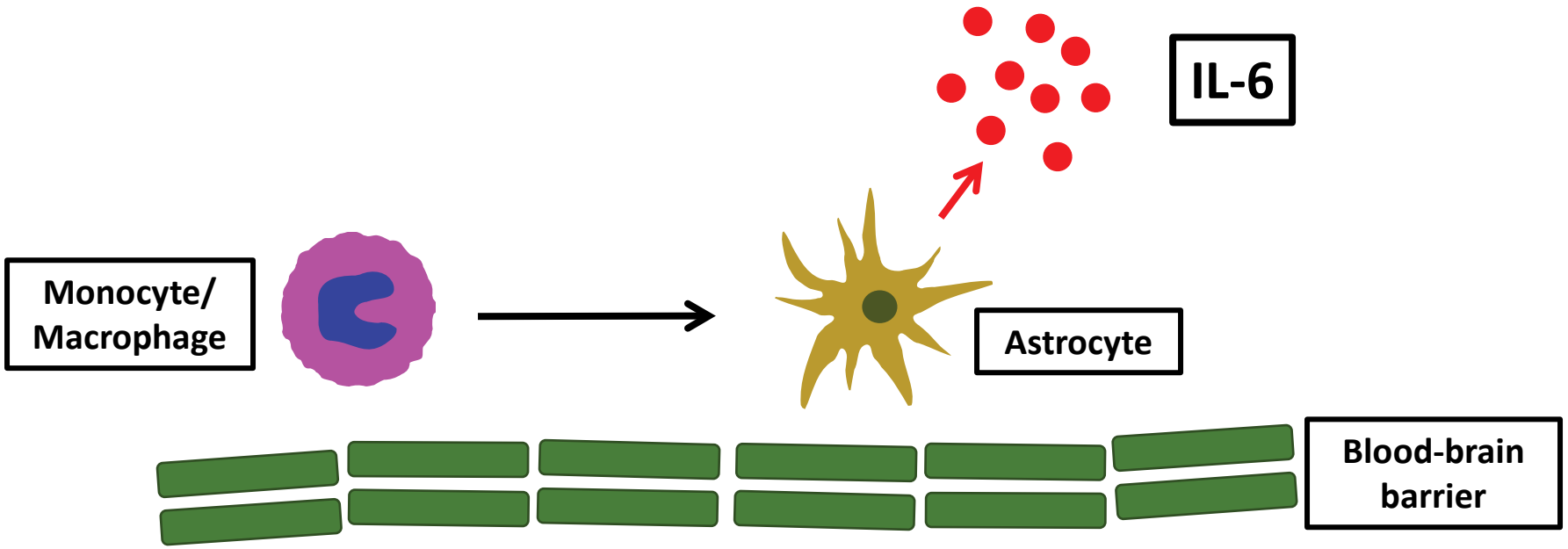
Endpoint measurements

24 hours

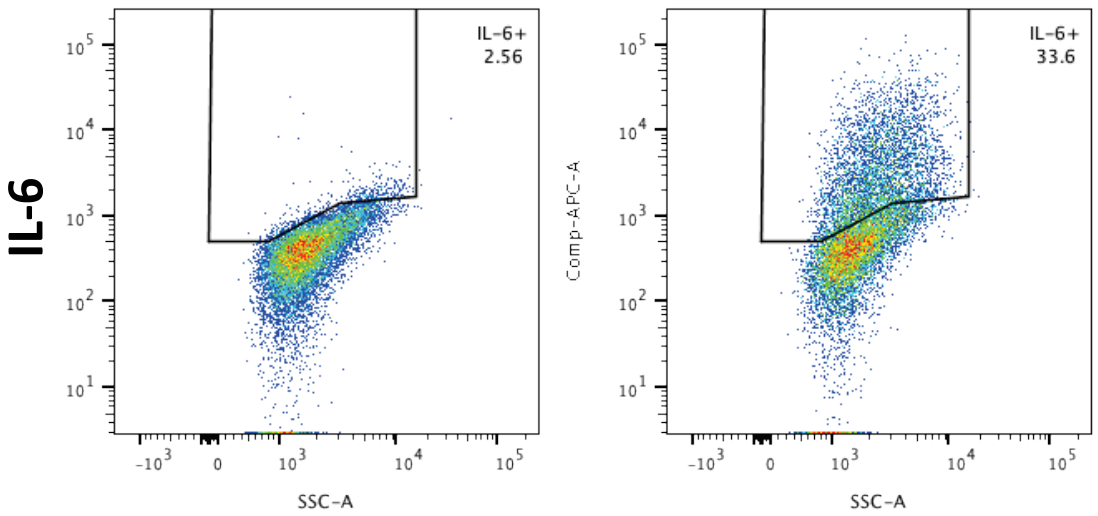


Endpoint measurements:
1. **Intracellular protein:** flow cytometry
2. **Supernatant protein:** LEGENDplex™ (bead-based)/ELISA

Objective: Determine if human monocytes promote astrocyte cytokine production (focus on IL-6, MCP-1 and IP-10 not shown)

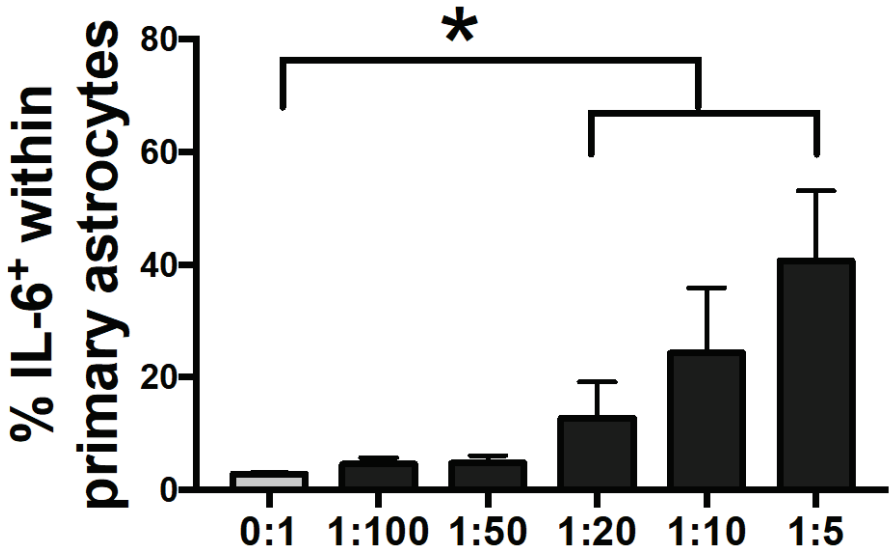


Monocytes Promote Astrocyte Production of IL-6



0:1 ratio **1:5 ratio**

Monocyte:Astrocyte ratio



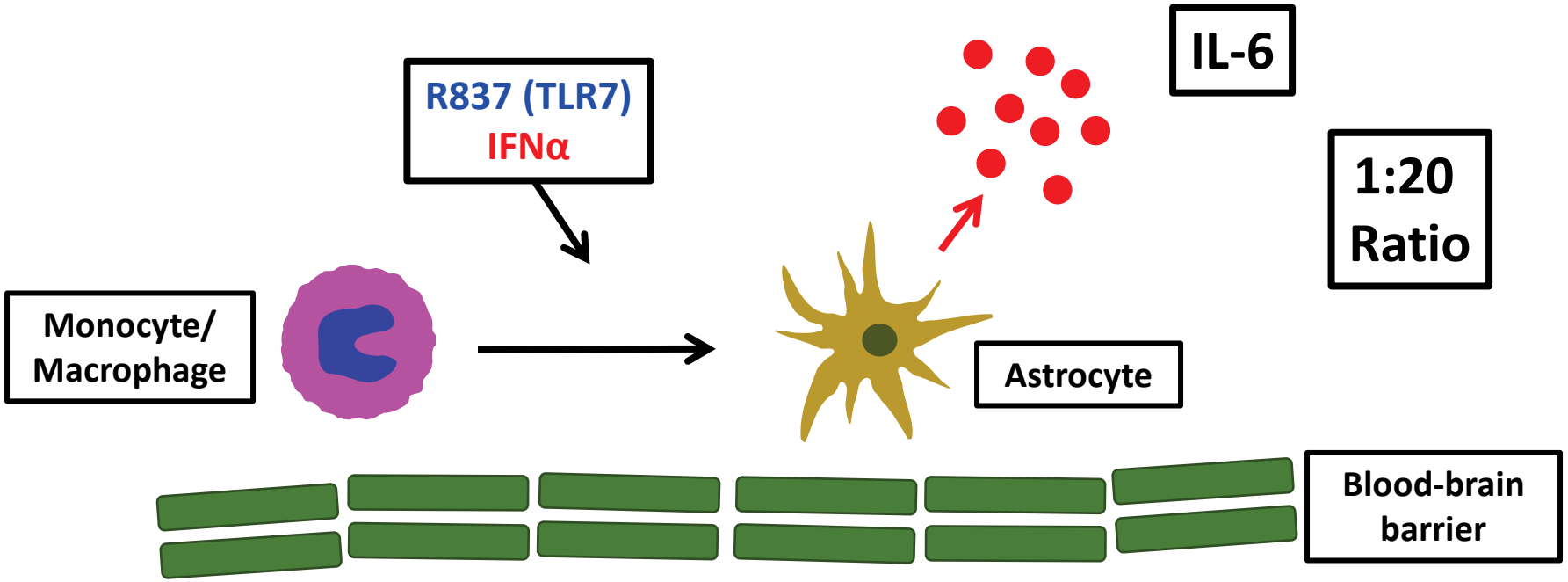
Cell Ratio (Monocyte: Astrocyte)

N=5 each for monocytes

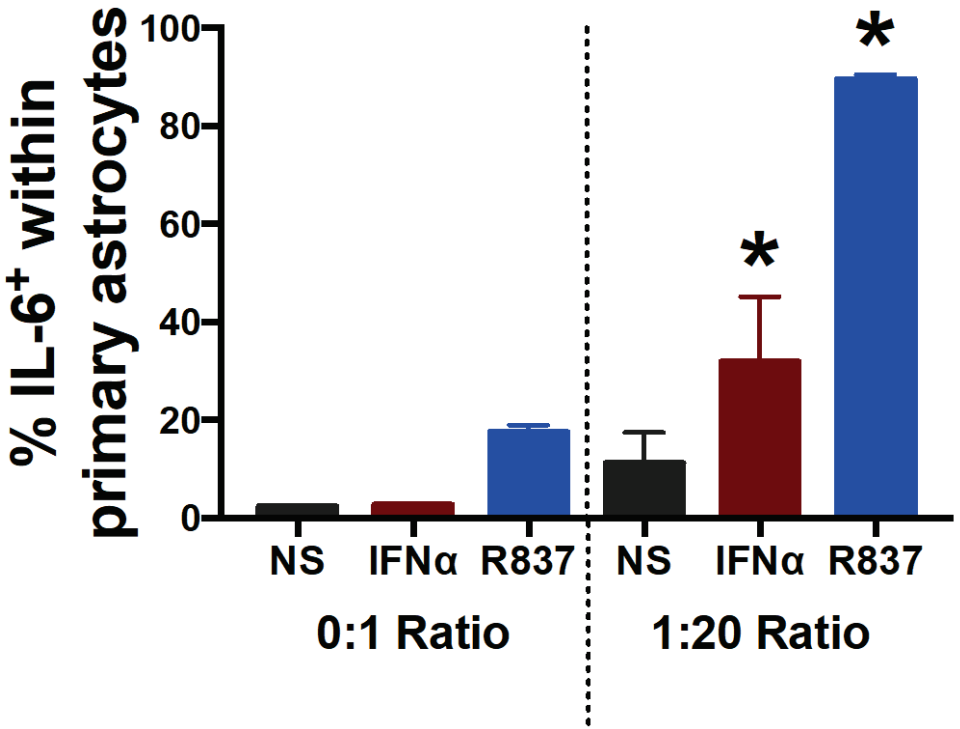
*Statistically different from 0:1 ratio ($p<0.05$)

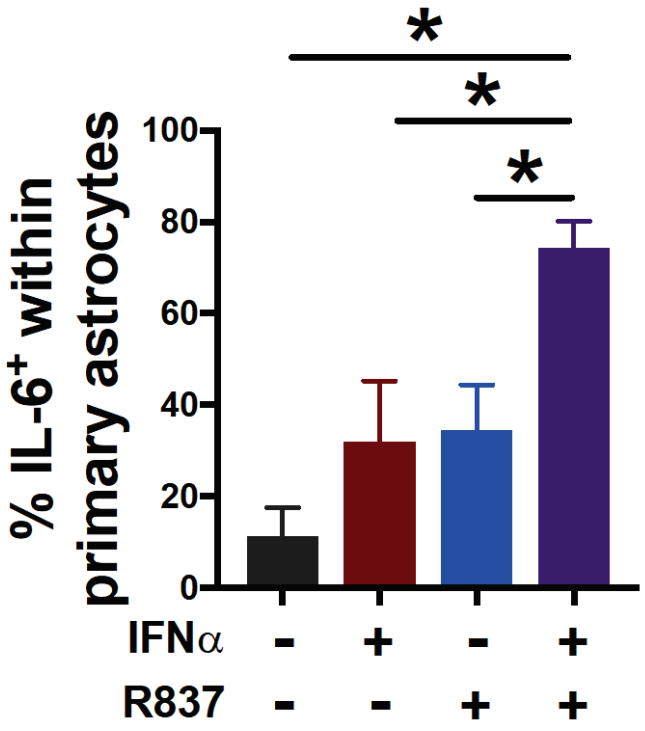
Objective: Determine if IFNα and TLR7 agonist (R837) augment monocyte-mediated astrocyte secretion of IL-6

HIV ssRNA – stimulates TLR7/8
IFNα – produced in response to HIV



IFNα and R837 treatment of co-culture enhances astrocyte production of IL-6



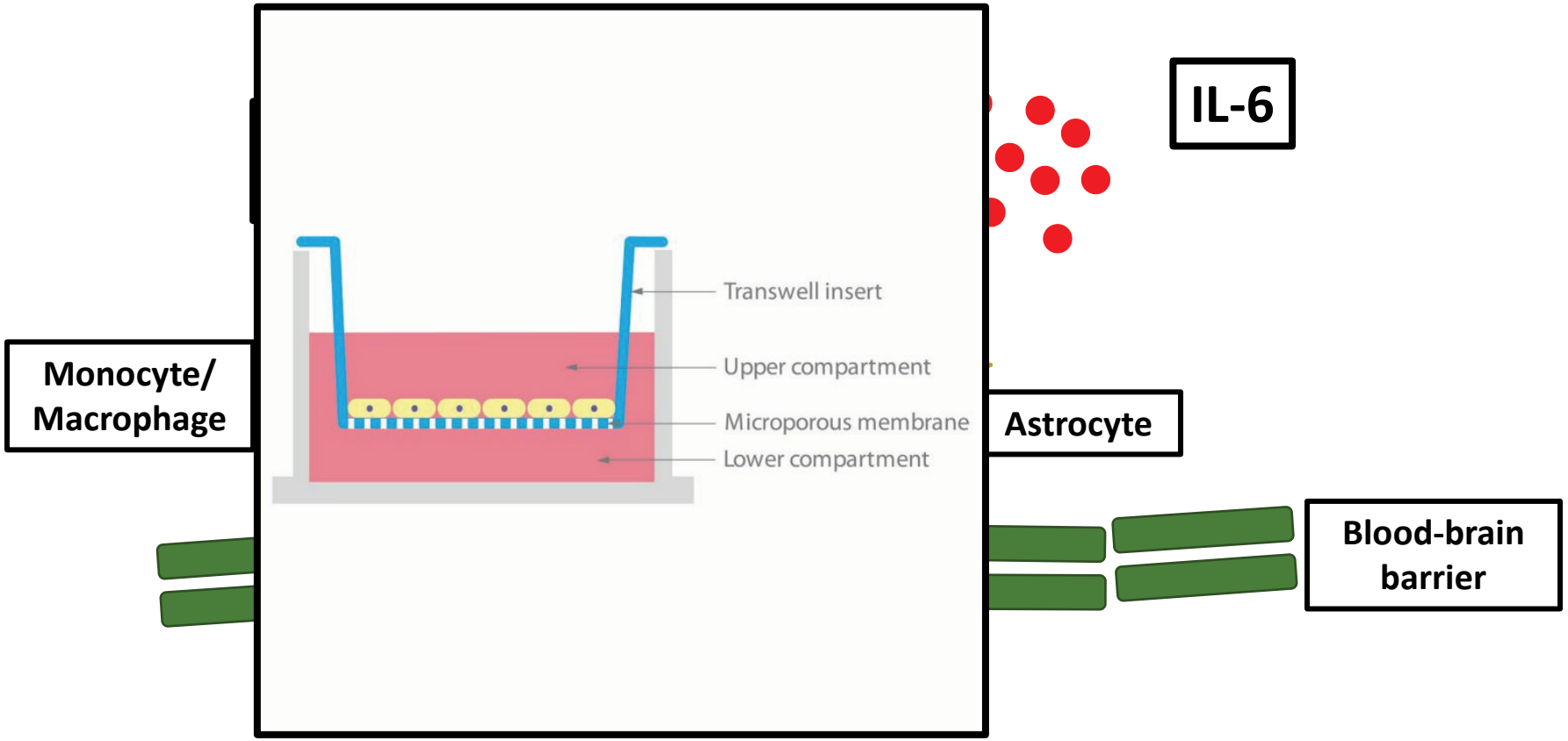


N=3-9 for monocytes (HIV-negative)

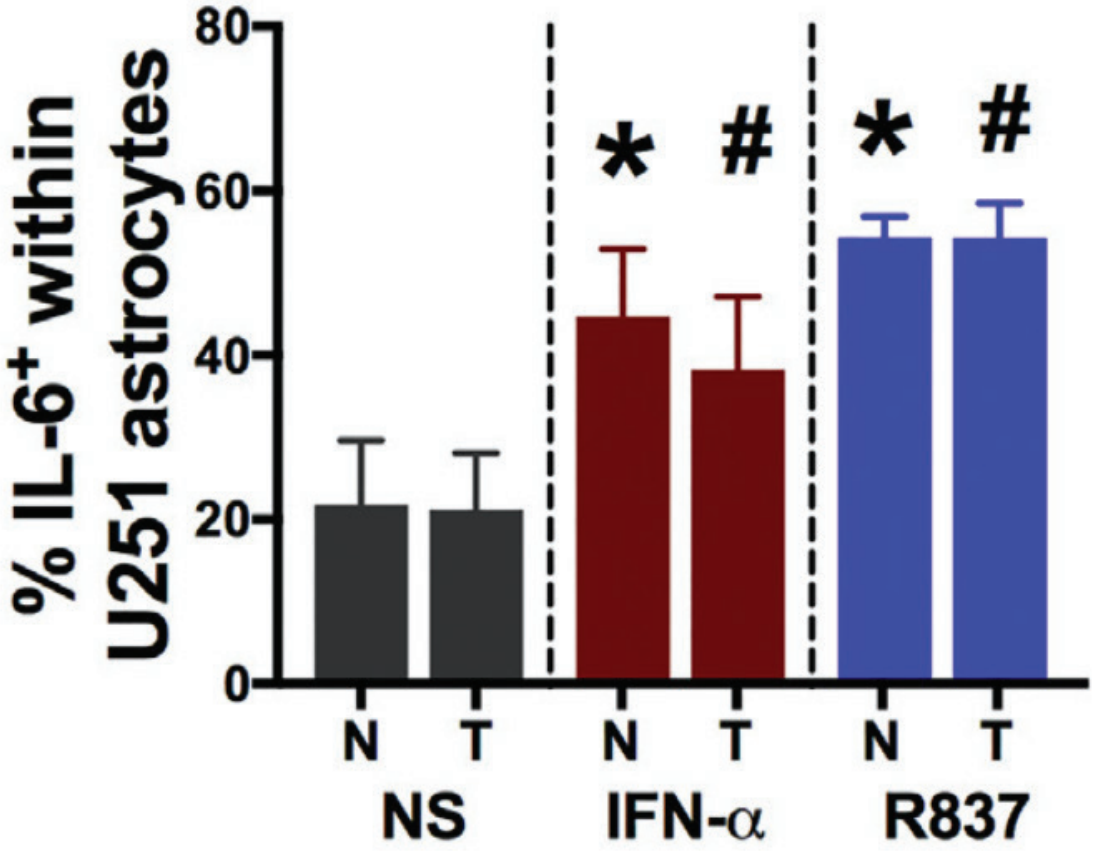
*Statistically different from non-stimulated (NS) of corresponding ratio ($p<0.05$)

Cell stimulation:
IFNα (100U/mL)
R837 (1µg/mL)

Objective: Determine the mechanism by which monocytes promote astrocyte secretion of IL-6



Monocyte-mediated Astrocyte Inflammation is Primarily Mediated by Soluble Factors



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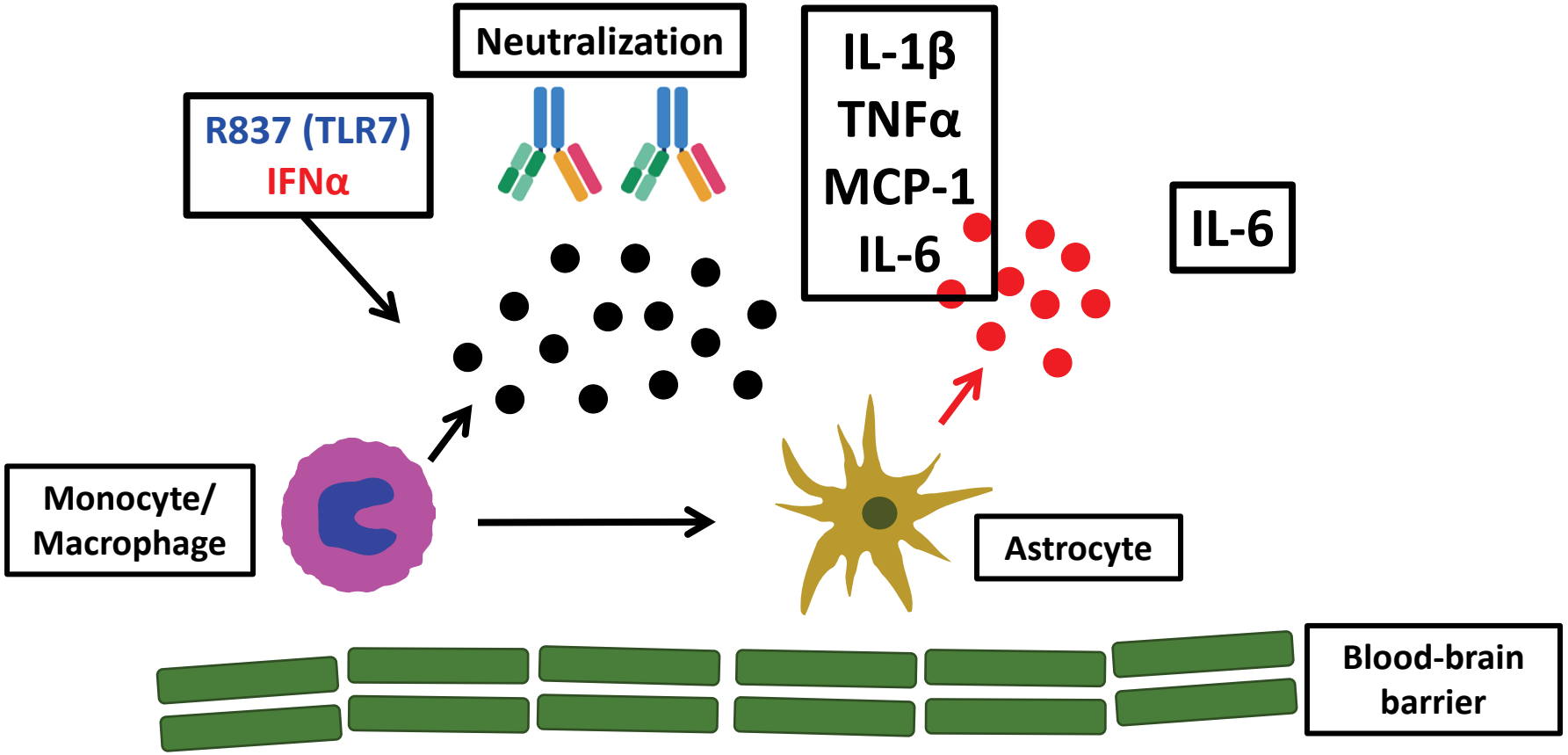

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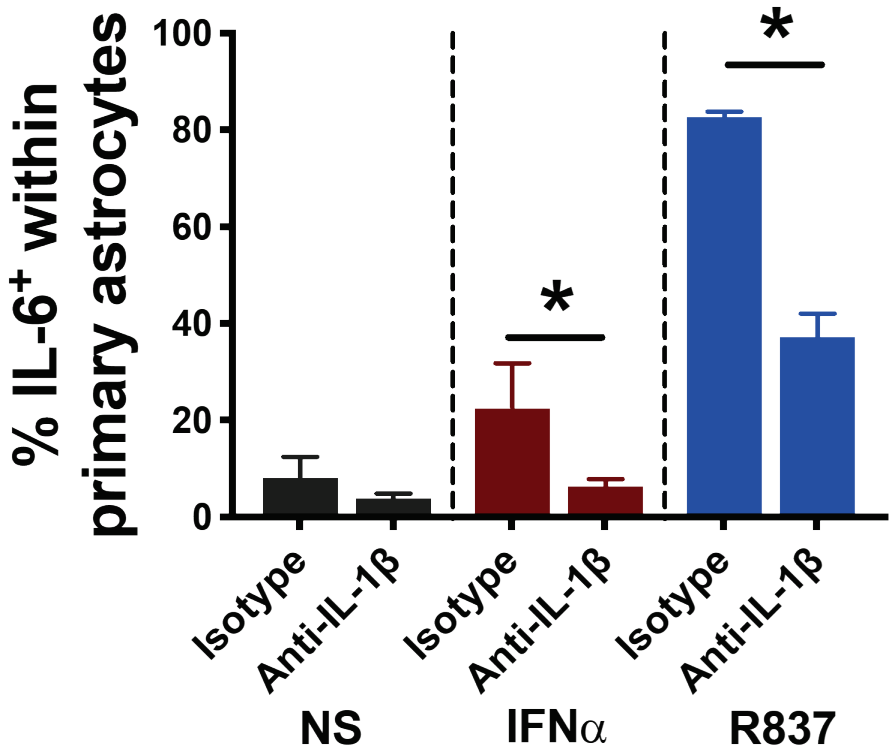
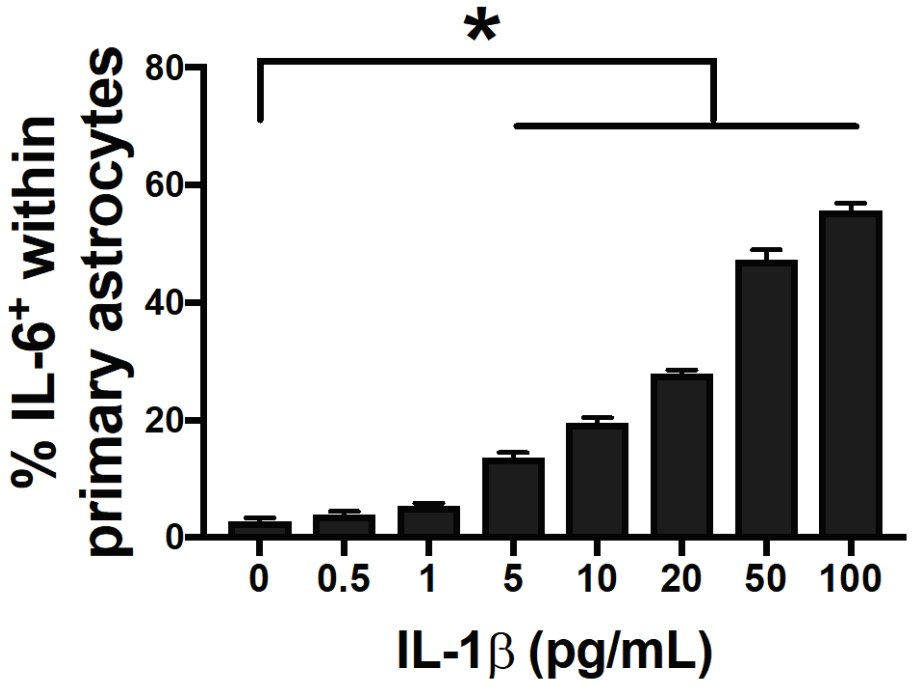


N=5-6 for monocytes (HIV-negative)
IFNα: 100U/mL
R837: 10μg/mL

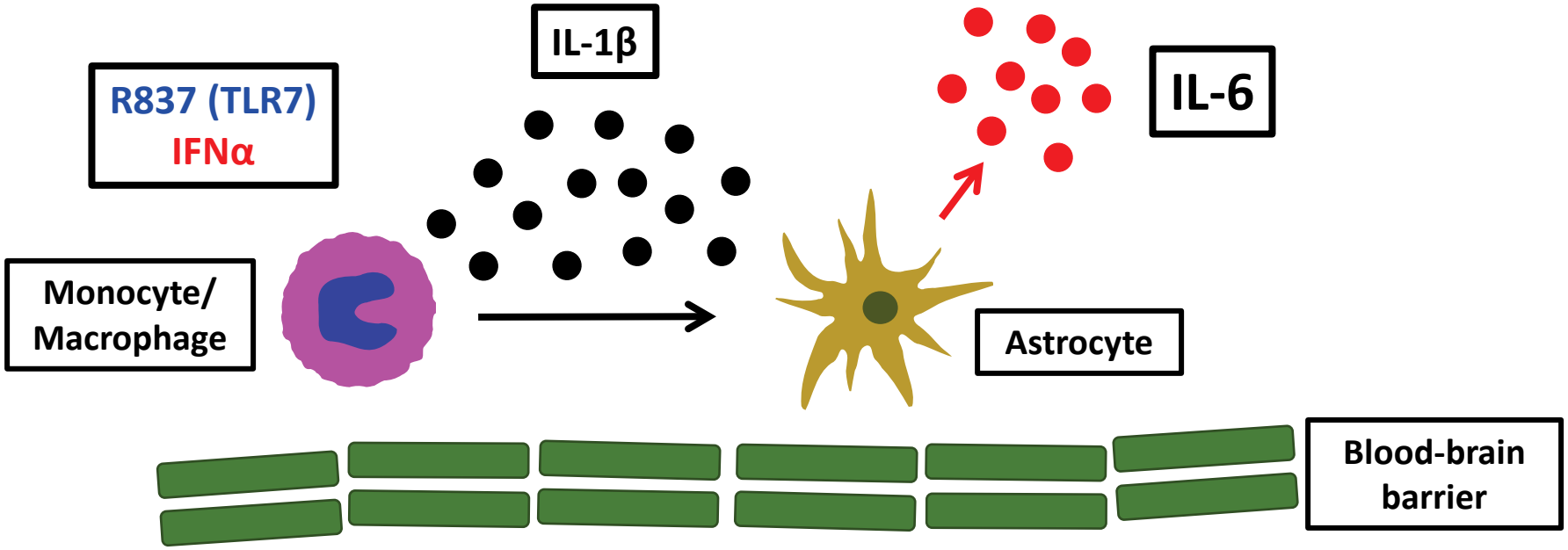
Recombinant IL-1β



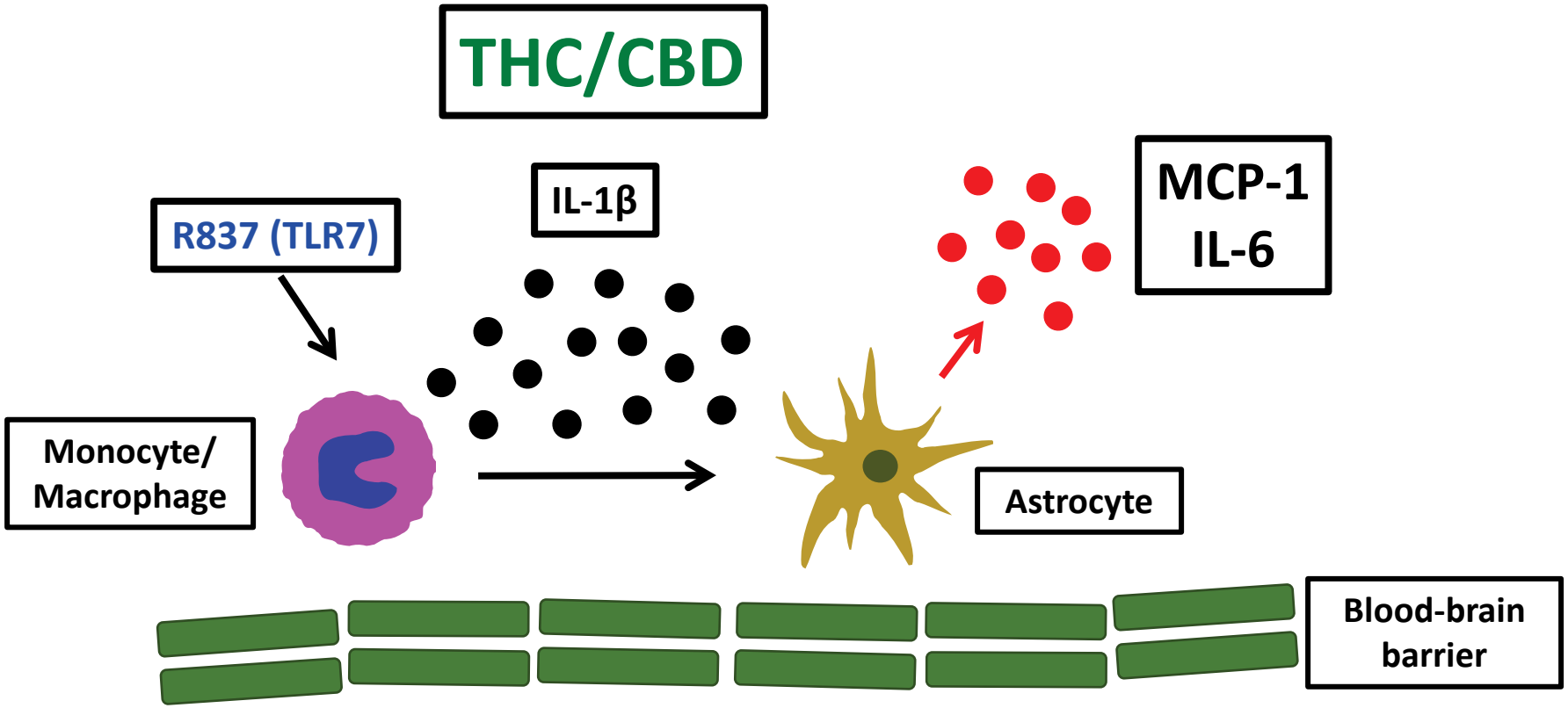
Rizzo *et al.* 2019

Summary

1. Monocytes promote astrocyte production of IL-6
2. IFNα and R837 (TLR7 agonist) augment astrocyte production of IL-6
3. Monocyte-derived IL-1β is an important factor driving astrocyte secretion of IL-6
4. May be an important mechanism contributing to HIV-associated neuroinflammation



Objective: Determine the effect of THC and CBD on monocyte-mediated astrocyte production of IL-6 when stimulated via TLR7

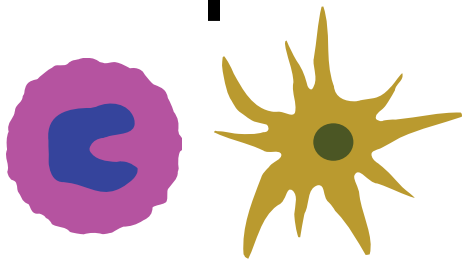


Human Monocyte-Astrocyte Co-culture: Experimental Design

Co-culture:
Cannabinoid treatment
TLR7/R837 (10µg/mL)
activation

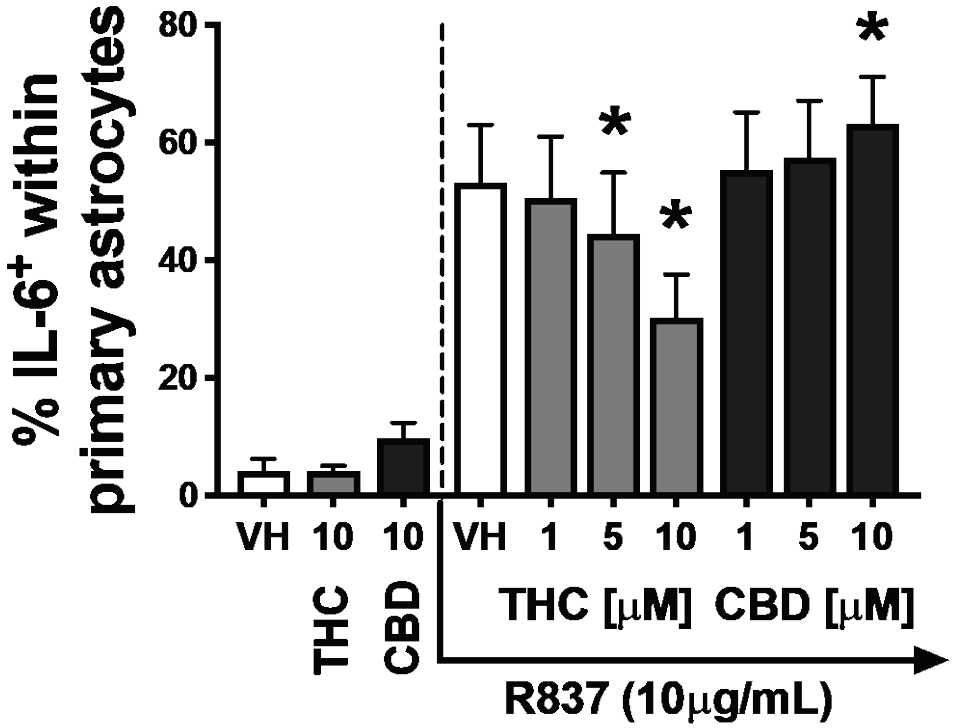
Endpoint
Measurements

24 hours



Endpoint measurements:
1. **Intracellular protein:** flow cytometry
2. **Supernatant protein:** LEGENDplex™ (bead-based)/ELISA

Differential Cannabinoid Modulation of Monocyte-mediated Astrocyte Production of IL-6 (Co-culture)



1:20 ratio

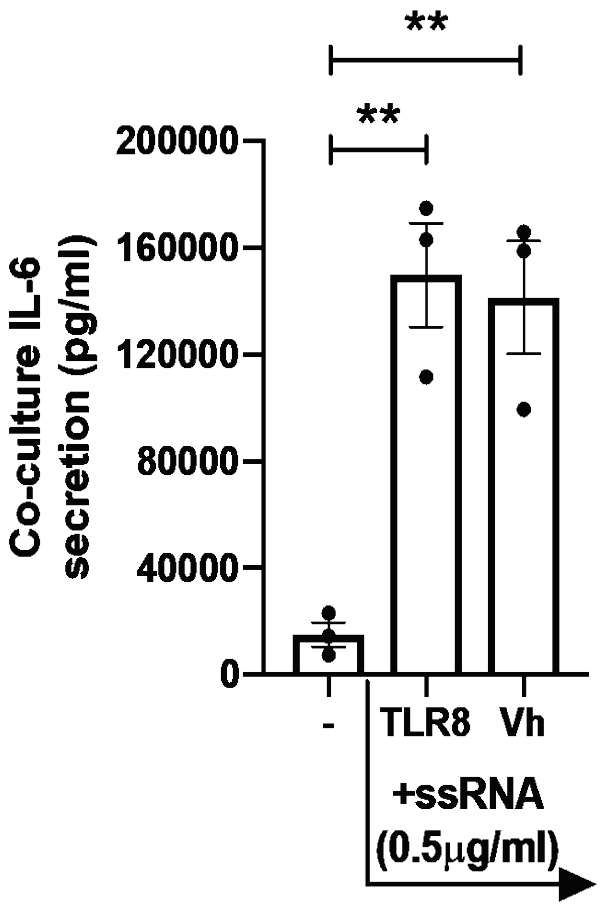
N=9 for monocytes (HIV-negative)
N=2 for primary astrocytes

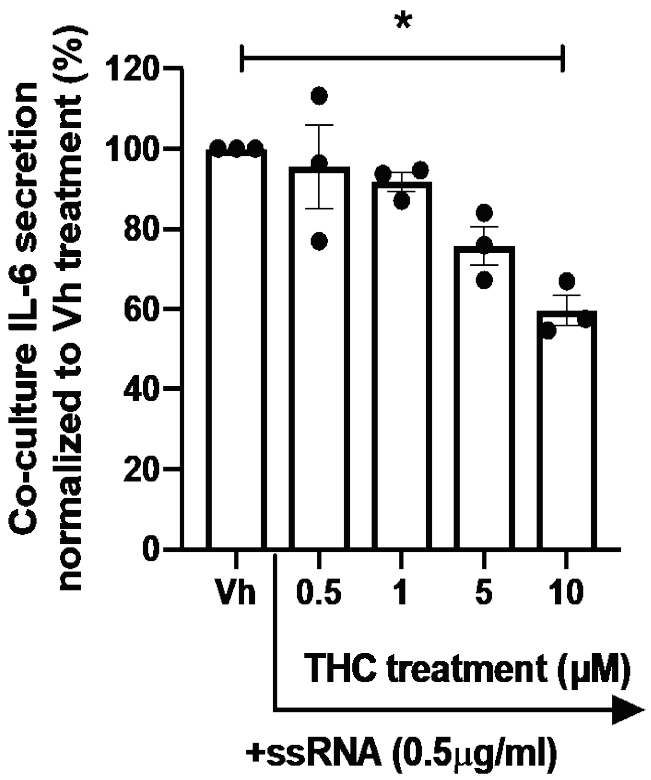
*Statistically different from R837-vehicle control ($p<0.05$)

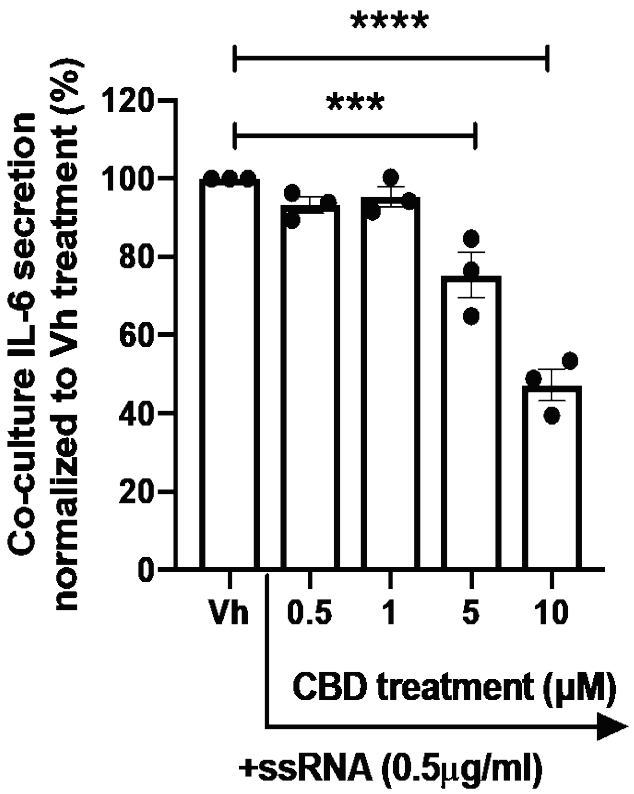
No effect on cell viability

Supernatant IL-6 levels in R837+VH: 59.7ng/mL

Cannabinoid Modulation of Monocyte-mediated Astrocyte Production of IL-6 (Co-culture)





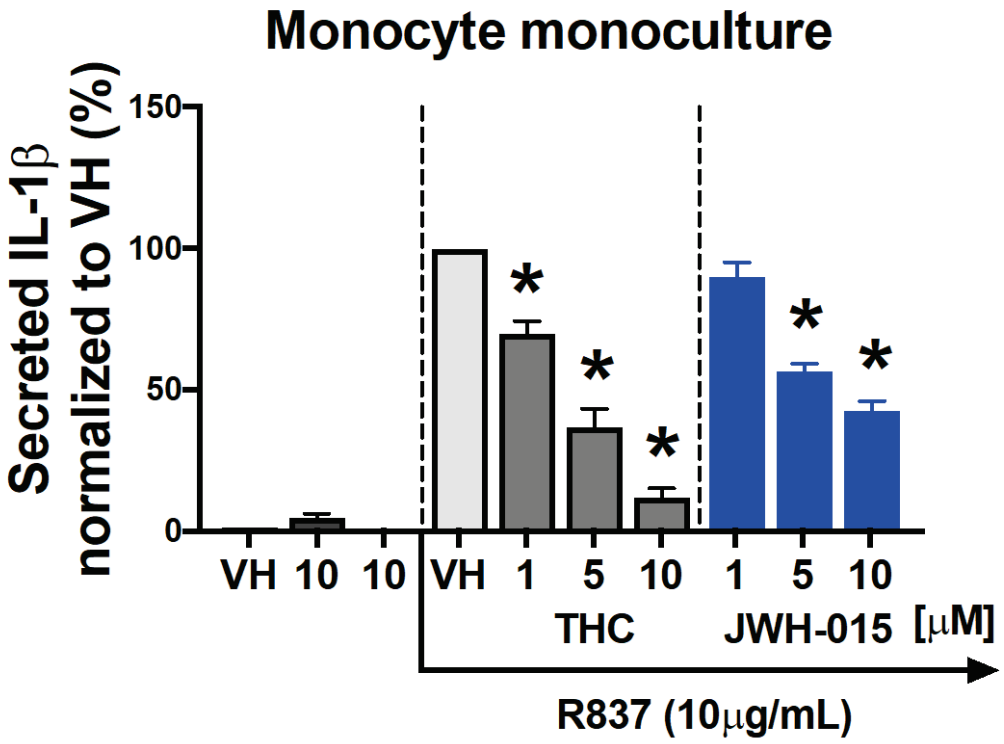


1:20 ratio

N=3 for monocytes (HIV-negative)
N=1 for primary astrocytes

The CB2 selective agonist, JWH-015, decreased TLR7-mediated monocyte secretion of IL-1β

Monocyte monoculture



N=12 for monocytes (HIV-negative)

*Statistically different from R837-vehicle control ($p<0.05$)

Conclusions

1. HIV-infected cannabis users display a lower level of activated $CD16^+$ monocytes compared to non-users
2. **<u>THC</u>** displays **immune suppressive** and **anti-inflammatory** activity on monocyte activation and monocyte-mediated astrocyte inflammatory responses
   - Monocyte transition to the $CD16^+$ phenotype
   - TLR7-stimulated monocyte-astrocyte co-culture inflammatory responses
   - TLR7-mediated secretion of IL-1β in monocytes from HIV+ donors
   - Astrocyte production of IL-6
3. **<u>CBD</u>** displays **differential** immune modulating activity, which is dependent on the stimulation and cell type
   - No effect on TLR7 (R837) induce IL-6 production by astrocytes
   - Impaired astrocyte production of IL-6 when stimulated ssRNA (TLR8) agonist
4. The CB2-selective agonist, **<u>JWH-015</u>**, suppresses TLR7-mediated monocyte secretion of IL-1β similar to THC

Significance

1. THC in cannabis may provide beneficial anti-inflammatory effects on monocyte processes implicated in HIV-associated neuroinflammation
   - Could be detrimental to other aspects of immune competence
2. Cannabinoid-based therapies may have differential effects on chronic neuroinflammation associated with HIV infection
   - THC promotes anti-inflammatory effects
   - CB2 selective agonists may have therapeutic application
3. Potentially applicable to other neurodegenerative diseases involving monocyte-driven inflammation
   - Multiple sclerosis (MS), Alzheimer's disease and systemic lupus erythematosus

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