Regulation of Inflammation by Cannabinoids

Prakash Nagarkatti, Ph.D.
Vice President for Research
Carolina Distinguished Professor
University of South Carolina
Columbia, SC
Introduction

Marijuana (Cannabis sativa):

- The oldest written record of use of cannabis dates back to 2727 B.C.—China and India
- Most widely used recreational drug worldwide
- Over 29 states have legalized medical marijuana use

2,700 year old Chinese tomb was discovered in 2008: Had 2 pounds of Cannabis
Marijuana (Cannabis sativa):

- Has over 400 chemicals. Contains >60 cannabinoids that act through cannabinoid receptors.

- **Major psychoactive component:**
  - $\Delta^9$ Tetrahydrocannabinol ($\Delta^9$ THC)

- **Non-psychoactive component:**
  - Cannabidiol
Marijuana (Cannabis sativa):

- **1611-1762** - Jamestown Settlers Bring Marijuana to North America
- **1840s** - Marijuana Becomes Mainstream Medicine in the West
- **1850** - Marijuana Added to US Pharmacopeia
- **1930s** - American Pharmaceutical Firms Sell Extracts of Marijuana as Medicines

Marijuana (Cannabis sativa):

- 1970 - Controlled Substances Act (CSA) Classifies Marijuana as a Drug with “No Accepted Medical Use”
- The CSA creates five schedules to classify substances
- Marijuana is placed in Schedule I: Drugs “classified as having a high potential for abuse, no currently accepted medical use in treatment in the United States, and a lack of accepted safety for use of the drug or other substance under medical supervision”
US Drug Enforcement Agency: Schedule I and II

Schedule I
- Heroin
- Ecstasy
- LSD
- Marijuana

No currently accepted medical use and a high potential for abuse

Schedule II
- OxyContin / OxyCodone
- Morphine
- Methadone
- Cocaine

High potential for abuse, potentially leading to severe psychological or physical dependence
Marijuana: Schedule I drug: Has no accepted medical use
US Govt holds patent No. 6630507 for marijuana to treat neurological diseases; anti-oxidant
FDA has approved Marinol: Synthetic THC to treat: nausea, vomiting: In cancer patients with chemotherapy, and to gain weight in HIV/AIDS
Sativex: THC&CBD. Approved in Europe, Canada to treat MS patients neuropathic pain and spasticity.
Epidolex=Cannabidiol: To treat epilepsy as an orphan drug
CBD approved by FDA to treat autoimmune hepatitis
Dependence Rates
National Institute on Drug Abuse

32% Tobacco
23% Heroin
17% Cocaine
15% Alcohol
9% Caffeine
9% Cannabis
Cannabinoid Receptors

CB1 receptor

CB2 receptor

Endocannabinoids

$\Delta^9$ THC

1980s

Immune System

Psychotropic Effects

Blood 100:627, 2001
J. Immunol. 174:3281, 2005
Cannabinoid receptors

- CB receptors evolved 600 million years ago.
- All vertebrates have them.
- CB receptors existed before cannabis plant evolved 25 million years ago.
Exo- and Endocannabinoid System

Endocannabinoids: Anandamide (AEA) 2-arachidonoyl glycerol (2AG)

Δ⁹ THC

CBD

• Memory
• Appetite
• Metabolic functions
• Thermoregulation
• Immune functions

The fatty acid amide hydrolase

Inflammation

Inflammation is a natural response from the immune system against infections or tissue injury.
Inflammation Homeostasis

**Effector**

**Pro-Inflammation**
- Innate: (Macrophage, Dendritic cells)
- Adaptive: (B, Th2, Th1, Th17)

**Regulatory**

**Anti-Inflammation**
- Innate: (Macrophages)
- Adaptive: (Th2, Tregulatory cells)

Infection  Immunity
Impact of Imbalance

Effector

Pro-Inflammation

↓

Chronic Inflammation

Regulatory

Anti-Inflammation
Inflammation: A Double-Edged Sword

- Inflammation is the underlying cause of all major clinical disorders.

1. Cardiovascular diseases
2. Neurodegenerative diseases
3. PTSD
4. Autoimmune diseases
5. Cancer
6. Obesity
7. Aging
Autoimmune Diseases
Over 80 disorders

- Arthritis
- Hashimoto’s thyroiditis
- Lupus
- Grave’s disease
- Myasthenia Gravis
- MS
Currently there is no cure for Autoimmune diseases
Can Cannabinoids Kill Activated Immune Cells?
Cannabinoid receptor activation trigger apoptosis (cell death)

Normal Cell  Apoptotic Cell

CB \(\rightarrow\) \(\rightarrow\) L R

DNA fragmentation

_Blood._ 2002 Jul 15;100(2):627-34
Autoimmune Hepatitis

- Autoimmune hepatitis (AIH): Affects all ages
- Prevalence, US: 100,000-200,000 people
- Frequency of AIH among patients with chronic liver disease in North America is 11-23%
- Clinical manifestations → fatigue; jaundice (~80%); hepatomegaly (78%); aspartate aminotransferase in blood (100%)
- Mechanisms: Mediated by T cells involving primarily Th1 cytokines and NKT cells.
THC treatment ameliorates ConA-induced hepatitis in mice
THC inhibits ConA-induced hepatitis

Analysis of liver enzymes in serum

Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)

0
500
1000
1500
2000
2500
3000

Asth (IU/L)

0h 6h 12h 24h
Vehicle THC ConA+Veh ConA+THC

**

**

**

**

16h
THC and Cannabidiol (CBD)

Psychoactive

Non-psychoactive

THC

CANNABIDIOL
Cannabidiol (CBD) treatment attenuates hepatitis

AST (IU/L)

- PBS
- ConA+Veh
- ConA+CBD (5mg/kg)
- ConA+CBD (20mg/kg)
- ConA+CBD (50mg/kg)
- CBD (50mg/kg)

- 6h
- 12h
- 24h
- 48h

* p<0.05, ** p<0.01

a) Vehicle;
b) ConA+Veh;
c) ConA+CBD (50mg/kg);
d) CBD alone
Revive Therapeutics Applies to FDA for Orphan Drug Designation for Treatment of Autoimmune Hepatitis

TORONTO, ONTARIO--(Marketwired - Sep 27, 2017) - Revive Therapeutics Ltd. ("Revive" or the "Company") (TSX VENTURE:RVV) (RVVTF), a company focused on the research, development and commercialization of novel treatments for serious and unmet medical needs, today announced that it has submitted an application to the U.S. Food and Drug Administration ("FDA") seeking orphan drug designation of cannabidiol ("CBD") for the treatment of autoimmune hepatitis ("AIH"), a rare liver disease.

"This orphan drug designation application is an important first step in the commercial development process of cannabidiol for the potential treatment of autoimmune hepatitis," said Craig Leon, Chief Executive Officer of Revive. "We are focused on advancing the research of novel therapies that target the endocannabinoid system, such as the CB1 and CB2 endocannabinoid receptors, and further strengthen our product pipeline with potentially safer and effective treatments for various liver diseases."

Under the Orphan Drug Act of 1983, the FDA provides incentives for companies developing treatments that are expected to provide significant therapeutic advantage over existing treatments and that target rare medical conditions affecting fewer than 200,000 U.S. patients per year. Incentives include seven-year market exclusivity, tax
MS & Experimental Autoimmune Encephalomyelitis (EAE)

Clinical Scores
0 No symptoms
1 Limp tail
2 Partial paralysis of hind limbs
3 Complete paralysis of hind limb or partial hind and front limb paralysis
4 Tetraparalysis
5 Moribund
6 Death

Multiple Sclerosis: Autoimmune disease of the central nervous system (brain & spinal cord)

MS patients

Day 0: MOG+CFA s.c.
2: PT i.p.
14: 2 PT i.p.
24:

Myelin oligodendrocyte glycopeptide (MOG$_{35-55}$)
Complete Freund’s Adjuvant (CFA)
Pertussis Toxin (PT)
Cannabidiol Can Suppress Multiple Sclerosis
Colitis

- Acute or chronic inflammation of the colon (large intestine)
- Inflammatory Bowel Diseases (IBD) – Crohn’s & ulcerative colitis

Population affected:
- All ages (frequent in 15-30 years old)
- No gender bias (males and females)

Complications:
- Abdominal pain/cramping
- Anorexia (weight loss)
- Diarrhea
- Gastrointestinal bleeding
- Nausea/Vomiting
- Sores (ulcerations)
- Higher risk for developing colon cancer

Treatments:
- Drug therapies to maintain and induce remission (immunosuppressive)
- Surgical removal of the colon and rectum (20 to 40 percent)
CB2 select agonist as anti-inflammatory agent: against colitis

THC protects mice from Septic Shock
Uveitis

- Inflammation of Uvea
- Triggered by infections, injury and autoimmune disorders
- Complications include: glaucoma, cataracts, fluid within the retina and vision loss
- One of the leading causes of blindness (10-15% of blindness due to uveitis)
- 38,000 new cases each year

Is cannabis effective against Cancer?

http://thenaturalwayofhealing.com/girl-eliminates-cancer/
http://www.medicalnewstoday.com/articles/299115.php
Do Cancer cells express cannabinoid receptors?

Normal Immune Cell

CB1
CB2

Gaβ3

Transformed Immune Cell

CB1
CB2

Gaβ3

Blood. 2002 Jul 15;100(2):627-34
Targeting CB2 cannabinoid receptors as a novel therapy to treat malignant lymphoblastic disease

Robert J. McKallip, Catherine Lombard, Michael Fisher, Billy R. Martin, Seongho Ryu, Steven Grant, Prakash S. Nagarkatti, and Mitzi Nagarkatti

Author Affiliations

View Full Text
Doctors, Patients Assess Effectiveness of Medical Marijuana

August 23, 2011 at 12:00 AM EDT
Cancer cells dying from apoptosis
Cancer cure in mice

Cancer cells

THC

Tumor Regression

Cured

Blood. 2002 Jul 15;100(2):627-34
Induction of Apoptosis in murine tumors by THC

$\Delta^9$ THC

CB1/CB2

Anandamide

2-AG

FAAH

DNA fragmentation

A

% of control cell number

\[\text{THC concentration (\text{\textmu M})}\]

EL-4

LSA

P815

B

Cell Number

Fluorescence Intensity

58%

26%

52%
THC Can Kill Human Leukemic cells

A

ALL no. 1

ALL no. 2

Cell Number x 10^6

Vehicle 1 5 10

THC concentration (μM)

Vehicle 1 5 10

Cell Number

Vehicle 1 5 10

Fluorescence Intensity

1 μM THC

5 μM THC

10 μM THC

B

7.1%

88.0%

79.3%

85.0%

75.1%

17.0%

66.7%

50%

40.2%

DOX-

Bad

MitoTR

Overlay
Bone marrow transplants

Used in certain types of cancers and genetic diseases, including:

- Acute leukemia
- Aplastic anemia
- Hemoglobinopathies
- Hodgkin's lymphoma
- Immune deficiencies
- Inborn errors of metabolism
- Multiple myeloma
- Myelodysplastic syndromes
- Non-Hodgkin’s lymphoma
- Plasma cell disorders
- POEMS syndrome
- Primary amyloidosis

Graft-Versus-Host Disease
Use of Cannabinoids to treat GVHD

Splenomegaly in mice with aGVHD

Day 4
Day 12
Day 21

DAY of GVHD
HISTOPATHOLOGY

THC treated GVHD mice showed significantly less infiltration

Control+PBS  
GVHD+PBS  
GVHD+THC

Liver

Colon
Treating Glioblastomas

- Glioblastomas are malignant brain tumors
- Very aggressive, difficult to treat

Do Glioblastomas express CB Receptors?


CBD kills Neuroblastoma

Vehicle

CBD
What are the mechanisms by which cannabinoids suppress inflammation?

Trends Pharmacol. Sci. 31:345, 2010
Mechanisms

- Cannabinoids Induce:
  - Apoptosis in activated immune cells
  - Switch from Th1 to Th2
  - Suppress inflammatory cytokines
  - Immunosuppressive
    - MDSCs
    - Tregs

Epigenetic Pathways
SEB-triggered inflammation: THC-mediated switch from Th1 to Th2

IFN-γ BALF (72 Hours)

SEB-triggered inflammation: THC-mediated switch from Th1 to Th2

IL-4 BALF

IL-10 BALF
Differentiation of CD4+ T cells

Thymus

Th0

- IL-12
- IL-4
- TGF-β (TGF-β)
- TGF-β (IL-6)

Th1
- T-bet; IFN-γ

Th2
- GATA-3; IL-4

Th17
- RORγt; IL-17

T_{reg}
- Foxp3; CD25^{+} IL-10/TGF-β

Pro-inflammatory

Anti-inflammatory
THC-induces FoxP3+CD4+ T regs
Myeloid-derived suppressor cells (MDSC)

- Heterogeneous, immature.
- Express granulocyte and macrophage markers
  - In mice, CD11b\(^+\)Gr1\(^+\)
- Potent suppressors - suppress T and NKT cells
- Promote T regs
- Produce iNOS, Arginase-1, IL-10
THC treatment leads to induction of MDSC

A  THC-induced MDSC express functional arginase

B  THC-induced CD11b+Gr-1+ cell migrate from BM and proliferate in periphery

Within the parietal peritoneum

J. Biol. Chem. 288:36810, 2013

Morphology: Giemsa stain
Epigenetic Regulation of gene expression

Diet/Nutrition

Stress

Environment
MicroRNA regulate expression of target genes

- Single stranded, noncoding small RNAs (19-25nts)
- Silence target gene
- Bind to 3’UTR mRNA
- Perfect- mRNA degradation
  Imperfect-translation inhibition
- Play a critical role in cellular differentiation and function
- Evolutionarily conserved
miRNA expression profile (microarray)
Expression of common and unique miR in various MDSCs
# Differentially expressed (Fold change) miRNA in THC-MDSC relative to control BM-MDSC precursors and other myeloid cells

<table>
<thead>
<tr>
<th>miRNA</th>
<th>THC-MDSC vs BM-MDSC</th>
<th>THC-MDSC vs SPL-MDSC</th>
<th>THC-MDSC vs SPL-MAC</th>
<th>THC-MDSC vs SPL-DC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Up-regulated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mmu-miR-690</td>
<td>6.76</td>
<td>3.03</td>
<td>5.76</td>
<td>18.85</td>
</tr>
<tr>
<td>mmu-miR-22</td>
<td>2.47</td>
<td>2.80</td>
<td>4.30</td>
<td>12.26</td>
</tr>
<tr>
<td>mmu-miR-361</td>
<td>2.42</td>
<td>2.35</td>
<td>3.56</td>
<td>8.99</td>
</tr>
<tr>
<td>mmu-miR-1195</td>
<td>2.35</td>
<td>2.16</td>
<td>2.98</td>
<td>6.69</td>
</tr>
<tr>
<td>mmu-miR-20a</td>
<td>2.21</td>
<td>-2.10</td>
<td>2.29</td>
<td>5.32</td>
</tr>
<tr>
<td>mmu-miR-875-3p</td>
<td>2.14</td>
<td>-2.12</td>
<td>2.16</td>
<td>4.41</td>
</tr>
<tr>
<td>mmu-miR-15b</td>
<td>2.06</td>
<td>-2.16</td>
<td>2.12</td>
<td>4.20</td>
</tr>
<tr>
<td>mmu-miR-27a</td>
<td>1.98</td>
<td>-2.44</td>
<td>2.01</td>
<td>4.02</td>
</tr>
<tr>
<td>mmu-miR-139-5p</td>
<td>1.97</td>
<td>-2.51</td>
<td>-2.31</td>
<td>3.55</td>
</tr>
<tr>
<td>mmu-miR-202-5p</td>
<td>1.96</td>
<td>-2.55</td>
<td>-3.28</td>
<td>3.52</td>
</tr>
<tr>
<td>mmu-miR-126-3p</td>
<td>1.90</td>
<td>-2.57</td>
<td>-3.31</td>
<td>3.29</td>
</tr>
<tr>
<td>mmu-miR-191</td>
<td>1.81</td>
<td>-2.65</td>
<td>-3.67</td>
<td>3.20</td>
</tr>
<tr>
<td>mmu-miR-23b</td>
<td>1.78</td>
<td>-2.69</td>
<td>-4.16</td>
<td>2.99</td>
</tr>
<tr>
<td>mmu-miR-491</td>
<td>1.78</td>
<td>-2.76</td>
<td>-4.33</td>
<td>2.63</td>
</tr>
<tr>
<td>mmu-let-7b</td>
<td>1.60</td>
<td>-2.83</td>
<td>-4.60</td>
<td>2.52</td>
</tr>
<tr>
<td>mmu-miR-503</td>
<td>1.57</td>
<td>-2.89</td>
<td>-4.94</td>
<td>2.50</td>
</tr>
<tr>
<td>mmu-miR-744</td>
<td>1.54</td>
<td>-3.09</td>
<td>-6.21</td>
<td>2.24</td>
</tr>
<tr>
<td>mmu-miR-130b</td>
<td>1.50</td>
<td>-3.11</td>
<td>-8.86</td>
<td>2.23</td>
</tr>
<tr>
<td>mmu-miR-204</td>
<td>1.50</td>
<td>-3.35</td>
<td>-10.18</td>
<td>2.08</td>
</tr>
<tr>
<td>mmu-miR-342-3p</td>
<td>-1.50</td>
<td>-4.01</td>
<td>-11.95</td>
<td>-2.34</td>
</tr>
<tr>
<td>mmu-miR-762</td>
<td>-1.62</td>
<td>-4.45</td>
<td>-5.32</td>
<td>-2.43</td>
</tr>
<tr>
<td>mmu-miR-378</td>
<td>-1.63</td>
<td>-4.60</td>
<td>-5.33</td>
<td>-2.73</td>
</tr>
<tr>
<td>mmu-miR-762</td>
<td>-1.67</td>
<td>-5.52</td>
<td>-6.54</td>
<td>-3.54</td>
</tr>
<tr>
<td>mmu-miR-342-3p</td>
<td>-1.97</td>
<td>-5.56</td>
<td>-8.68</td>
<td>-3.58</td>
</tr>
<tr>
<td>mmu-miR-335-5p</td>
<td>-3.33</td>
<td>-5.98</td>
<td>-10.18</td>
<td>-3.59</td>
</tr>
<tr>
<td>miR-762</td>
<td>0.71</td>
<td>-7.28</td>
<td>-11.95</td>
<td>-5.72</td>
</tr>
<tr>
<td><strong>Down-regulated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mmu-miR-876-3p</td>
<td>-1.50</td>
<td>-9.52</td>
<td>-13.83</td>
<td>-8.66</td>
</tr>
<tr>
<td>mmu-miR-719</td>
<td>-1.62</td>
<td>-14.46</td>
<td>-19.90</td>
<td>-10.18</td>
</tr>
</tbody>
</table>
Ingenuity Pathway analysis of genes targeted by differentially expressed miRNA in THC-MDSC
### Summary of miRNAs involved in myeloid cell development, differentiation and function: Role of miR-223

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Expressed in</th>
<th>Regulated by</th>
<th>Targets</th>
<th>Function</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-9</td>
<td>Myeloid cells</td>
<td>CREB</td>
<td>NF-κB1, ETS1, BCL6</td>
<td>Increased expression negatively regulates the acute responses following immune activation by downregulating proteins involved in signaling pathways</td>
<td>33,45,46</td>
</tr>
<tr>
<td>miR-17-5p</td>
<td>Myeloid cells</td>
<td>AML-1</td>
<td>AML-1</td>
<td>In combination with miR-20a and miR-106a, miR-17-5p down regulates M-CSF receptor expression and enhances blast proliferation and inhibits monocyte differentiation and maturation</td>
<td>9,14</td>
</tr>
<tr>
<td>miR-196b</td>
<td>HSC myeloid cells</td>
<td>MLL, GFI-1</td>
<td>HOX</td>
<td>Increases expression in ST-HSC and promotes lineage commitment of MPP. Its overexpression in lineage progenitors increases their proliferation and survival and blocks differentiation</td>
<td>34,47</td>
</tr>
<tr>
<td>miR-20a</td>
<td>Myeloid cells</td>
<td>AML-1</td>
<td>AML-1</td>
<td>Same as miR-17-5p</td>
<td>9,14</td>
</tr>
<tr>
<td>miR-21</td>
<td>Myeloid cells</td>
<td>GFI-1</td>
<td>NFI-β, PTEN</td>
<td>Overexpression in myeloid progenitors blocks G-CSF-induced granulocytic differentiation</td>
<td>47</td>
</tr>
<tr>
<td>miR-27</td>
<td>Myeloid cells</td>
<td>C/EBPα</td>
<td>AML-1</td>
<td>Overexpression in granulocytic progenitors downregulates AML-1 and promotes their differentiation</td>
<td>48,49</td>
</tr>
<tr>
<td>miR-29a</td>
<td>HSC, early progenitors</td>
<td>C-MYC</td>
<td>CDC42, HBP1</td>
<td>Increased expression in HSC and downregulated in progenitors.</td>
<td>35,50,51</td>
</tr>
<tr>
<td>miR-106a</td>
<td>Myeloid cells</td>
<td>AML-1</td>
<td>AML-1</td>
<td>Induces self-renewal capacity of HSC</td>
<td>9,14</td>
</tr>
<tr>
<td>miR-126</td>
<td>HSC, early progenitors</td>
<td>ETS1/2</td>
<td>HOX9A</td>
<td>Promotes generation of lineage-restricted progenitors (CLP &amp; CMP) from HSC</td>
<td>35,51,52</td>
</tr>
<tr>
<td>miR-155</td>
<td>Myeloid, B- and T-cells</td>
<td>AP-1</td>
<td>PU.1, C/EBP-β</td>
<td>Overexpression in HSC promotes proliferation and produce myeloproliferative disorders similar to acute myeloid leukemia</td>
<td>18,26,53</td>
</tr>
<tr>
<td>miR-223</td>
<td>Myeloid cells</td>
<td>C/EBPα, NFI-A</td>
<td>NFI-A</td>
<td>Negative regulation of neutrophil proliferation and activation (that is, induces their differentiation)</td>
<td>54,55,56</td>
</tr>
<tr>
<td>miR-424</td>
<td>Myeloid cells</td>
<td>PU.1</td>
<td>NFI-A</td>
<td>Controls M-CSFR expression. Increased expression in monocytic progenitors stimulates their differentiation</td>
<td>57</td>
</tr>
</tbody>
</table>

Abbreviations: AML-1, acute myeloid leukemia-1; C/EBPα, CCAAT/enhancer-binding protein; CLP, common lymphoid progenitors; CMP, common myeloid progenitors; HSC, hematopoietic stem cell; G-CSF, granulocyte colony stimulating factor; GFI-1, growth factor independent 1; M-CSFR, macrophage colony stimulating factor receptor; MiRNAs, microRNAs; MPP, multipotent progenitor; NFI-A, nuclear factor I-A; NFI-β, nuclear factor I-β; ST, short-term.
Myeloid regulator miR-223

Mmu-miR-223 target genes (TarBase 6.0)

- Mef2c
- Igf1r
- C330002I19Rik
- Tspyl3
- Ppp4r2
- Hpcal4
- Pcdh17
- Ankrd17
- Stad13
- Gpr158
- Atg4d
- Pdia6
- Gria3
- Cckbr
- Ywhaz
- Pdck1
- Pik3r3
- Gtlf3b
- Tom12
- Trip12
- Tmod2
- Ttc4
- Ankra2
- Ap2b1
- Slc8a1

miR-223 sequence with 3’ UTRs of target genes Igf1r and Mef2c mRNAs

Mouse Chromosome X Linkage Map (Mouse Genome Browser) showing the position of microRNA gene mmu-miR-223
Role of miR-223 in the regulation of MDSCs

Hematopoietic stem cells
Myeloid precursors + Tumor Antigens

miR-223

miR-223

THC

Transcription factor
Mef2c

miR-223

MDSCs

Th1 to Th2 switch: THC-induced hypomethylation of IFN-γ and hypermethylation of IL-4 and IL-10 promoters

Histone modification

Ac: acetylation
Me: methylation
Ub: ubiquination
Su: sumoylation
P: phosphorylation
Genome-wide Histone methylation and gene expression in THC treated activated T lymphocytes

J. Biol. Chem. 289:18707, 2014
J. Biol. Chem. 201:15460, 2016
Th1 to Th2 Switch: Histone methylation

K4, K36: Activator
K27, K9: Repressor

Th1 Transcription factor
Microbiome

- 100 trillion microbes present in the intestines
- Play a critical role in regulating inflammation
- Microbiota are identified by 16S rRNA V3 and V4 sequencing
- Dietary fibers are fermented to short-chain fatty acids (SCFAs) by microbes
Alpha and Beta Diversity in microbial composition in colon after SEB

- **Naïve**
- **SEB+THC**
- **SEB+Vehicle**

**Graph:**
- **X-axis:** Sequences Per Sample
- **Y-axis:** Rarefaction Measure: chao1
- **Legend:**
  - Red: Naïve
  - Blue: THC
  - Orange: Vehicle

**Scatter Plot:**
- **PC1:** 42.29%
- **PC2:** 11.14%
- **PC3:** 10.16%
- **Clusters:** Naïve, SEB+Vehicle, SEB+THC

**Colon**
Colonic microbiota in SEB-induced ALI following THC treatment (Phylum level)
Colonic microbiota in SEB-THC-treated mice (Class level)
Colonic microbiota in SEB-THC-treated mice (Order)

Sphingomonadales
Rhizobiales
VS2
Bifidobacteriales
Verrucomicrobiales
Enterobacteriales
RF32
Anaeroplasmatales
Actinomycetales
Desulfovibrionales
Burkholderiales
Bacteroidales
Actinomycetales
Chlorobiales
Acidobacteria
cWAS
Bacillales
Deferribacteres
Clostridiales
RF36
Erysipelotrichales
Lactobacillales
VS2
Bacteroidales
Caulobacterales

**Burkholderiales OTUs %**

<table>
<thead>
<tr>
<th></th>
<th>Naive</th>
<th>SEB+Vehicle</th>
<th>SEB+THC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Naive</strong></td>
<td>0.0</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>SEB+Vehicle</strong></td>
<td>0.6</td>
<td>0.8</td>
<td><strong>0.8</strong></td>
</tr>
<tr>
<td><strong>SEB+THC</strong></td>
<td>0.8</td>
<td>0.8</td>
<td><strong>0.8</strong></td>
</tr>
</tbody>
</table>

* * *
Colonic microbiota in SEB-THC treatment (Family level)
Colonic microbiota in SEB-THC (Genus level)
Colonic microbiota in SEB-induced ALI following THC treatment (species level)

**Bacteroides** induce Tregs—anti-inflammatory
Summary

- Immune cells and cancer cells express CB receptors
- Cannabinoids are potent anti-inflammatory and anti-cancer agents
- More research and clinical trials need to be carried out

- The most prestigious scientific association in the US.
- Reviewed >10,000 publications in the field of cannabis.
- Highly effective against:
  - Chronic pain
  - Chemotherapy-induced nausea and vomiting in cancer patients
  - Multiple sclerosis (MS)
Acknowledgements

Dr. Mitzi Nagarkatti
Dr. Narendra Singh
Dr. Xiaoming Yang
Dr. Udai Singh
Dr. Elizabeth Zumbrun
Dr. Venkatesh Hegde
Dr. Brandon Busbee
Dr. Jessica Sido
Dr. Alexa Gandy
Dr. Marpe Bam
Dr. Chitrala Naidu
Dr. Farhan Zameer
Dr. Hasan Alghetaa
Pegah Mehrpooya
William Becker
Kathryn Miranda
Esraah Alharris
Haider Alrafas
Muthanna Sultan
Osama Abdulla
Wurood Neamah
Zinah Al-Ghezi
Amira Mohammed
Lorraine Madur
Yin Zhong

NIH grants P01AT003961, P20GM103641, R01AT006888, R01MH094755, R01AI123947, and R01AI129788
Thank you