In vitro Effects of CBD on SEB Activated Splenocytes

SMART 2021

Alanna Langford, Kiesha Wilson, Xiaoming Yang

Department of Pathology, Microbiology and Immunology, University of South Carolina School of Medicine, Columbia, SC, USA

UNIVERSITY OF SOUTH CAROLINA

BACKGROUND

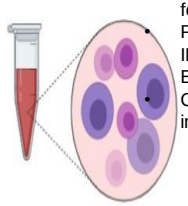
- Staphylococcal Enterotoxins are known as being major human pathogens, causing numerous infections ranging from skin infections to toxin-mediated diseases. These toxins are heat-stable and have a profound effect on the intestinal tract, hence they are commonly the cause of food poisoning
- SEB is a prototype of non-egc-associated potent SAg, and falls in the category B because it is the most potent staphylococcal enterotoxin
- SEB targets T cells and MHC class II molecules on the antigen presenting cells, after activation of transcriptional factors, a cytokine "storm" is produced including IL-1 and TNF-α from macrophages
- CBD (cannabidiol) have been shown to target mononuclear cells by suppressing TNF-α and IL-1α, and can also induce apoptosis in monocytes
- CBD has also been shown to target macrophages in animal models

METHODS

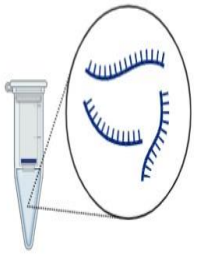
- Spleen cell isolation
- Treated cells with - an activator SEB (3.5 micro liters) and a treatment CBD (220 micro liters

Cultured for 48 hours at 1 x 10^6 per well in a 24 well plate

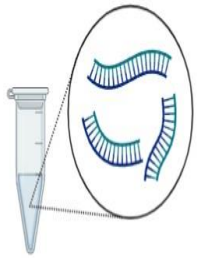
- Collected cells via centrifugation and saved supernatants for ELISA
- Performed IL1B and IL6 ELISA
- Cells frozen in Qiazol



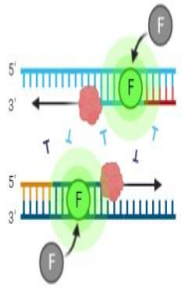
RNA isolated using Qiagen RNeasy isolation kit



Synthesized cDNA



Performed qPCR



RESULTS

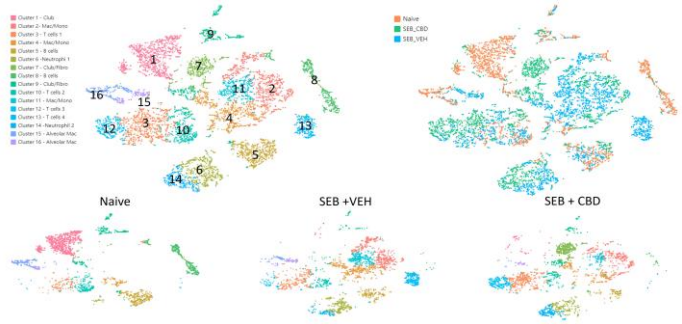
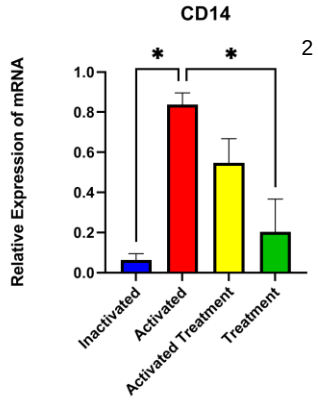


Fig. 1. A tSNE of single cell RNA sequencing



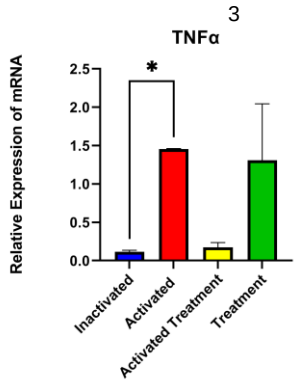
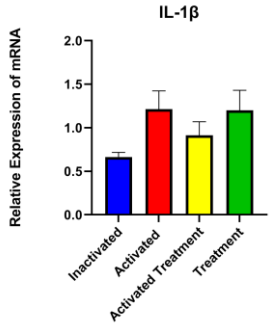


Fig 2-3. Gene expression levels of CD14 and TNF-α in different samples activated with SEB and treated with CBD



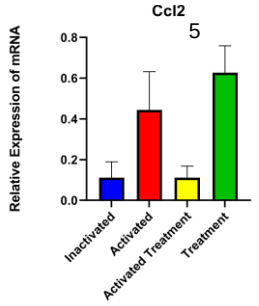


Fig 4-5 Gene expression levels of IL-1β and Ccl2 in different samples activated with SEB and treated with CBD.

RESULTS

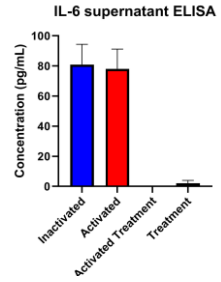


Fig 6. cytokine concentrations of IL-6 in each sample

CONCLUSIONS

- IL-6 cytokine concentrations were lower in the activated samples treated with CBD
- From the PCR, there was a significant difference in the CD14 gene expression were lower in activated samples treated with CBD the treatment samples.
- TNF-α expression was also lower in samples in activated samples treated with CBD.
- There was little expression of IL-1β in the samples

FUTURE DIRECTIONS

- Repeat invitro experiments
- Evaluate RNA expression of cell markers for different immune cells types
- Determine if macrophage populations are similar in severe COVID-19 reactions and SEB immune reactions

REFERENCES

Fries, B. C., & Varshney, A. K. (2013). Bacterial toxins—Staphylococcal enterotoxin B. *Microbiology spectrum*, *1*(2), 1-2.

Nichols, J. M., & Kaplan, B. L. (2020). Immune responses regulated by cannabidiol. *Cannabis and cannabinoid research*, *5*(1), 12-31.

Warrington, R., Watson, W., Kim, H. L., & Antonetti, F. R. (2011). An introduction to immunology and immunopathology. *Allergy, Asthma & Clinical Immunology*, *7*(1), 1-8.

ACKNOWLEDGEMENTS

SMART research funding from the Office of Undergraduate Research with a special thanks to Dr. Kiesha Wilson, Alkeiver Cannon, Bryan Holloman, Dr. Mitzi