

TREM2 regulates the phagocytic capabilities of HMC3 cells; implications for HIV associated neurocognitive disorders

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ABSTRACT

Goals of this Study/Hypothesis: Despite effective antiretroviral therapy and reduced viral loads, HIV-associated neurocognitive disorder (HAND) persists, and the underlying mechanisms are unknown. Recent studies suggest that cannabis may be neuroprotective in HAND by reducing inflammation. Microglia play an important role in neuroinflammation and exist on a spectrum of phenotypes ranging from pro-inflammatory and toxic (M1) to anti-inflammatory and neuroprotective (M2). The M2 phenotype is associated with increased levels of triggering receptor expressed on myeloid cells 2 (TREM2), increased amyloid beta (A β) phagocytosis, and reduced neurodegeneration. We proposed that HIV relevant stimuli (HRS) would induce the M1 phenotype and reduce TREM2 expression, and a cannabinoid receptor agonist would reverse these changes.

Materials & Methods: A human microglial cell line, HMC3, was used as a model. First, we exposed the microglia to HRS and measured levels of TREM2 and cytokines by western blot and real-time quantitative reverse transcription PCR (RT-qPCR). In addition, we measured the microglia phagocytic ability using fluorescein labeled A β . We repeated this experiment by inducing or knocking down TREM2, using a cannabinoid receptor agonist and siRNA respectively.

Results: HMC3 cells treated with HRS exhibited reduced TREM2 and increased inflammatory cytokine levels compared to controls. Furthermore, siRNA knockdown of TREM2 in these same cells significantly decreased phagocytosis of A β compared to controls. Lastly, a cannabinoid receptor agonist increased HMC3 TREM2 expression levels.

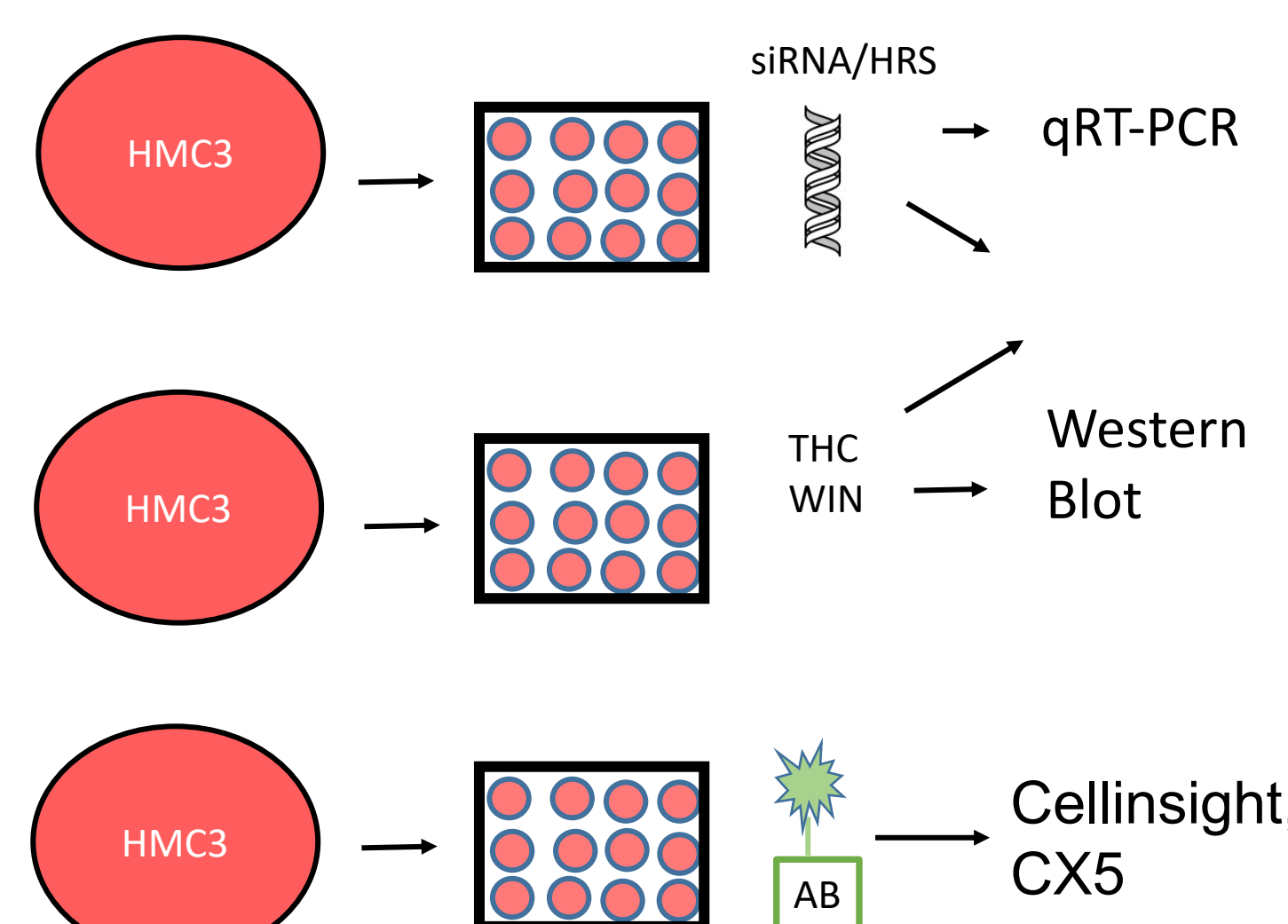
Conclusions: HRS reduces TREM2 and increases proinflammatory cytokines in HMC3s, inducing the toxic M1 phenotype. The decreased phagocytosis by HMC3s with TREM2 knockdown identifies TREM2 as an important regulator in microglial phenotype. Thus, maintaining TREM2 expression levels could be targeted to promote the protective phenotype of microglia during HIV infection. Lastly, we show that cannabinoid receptor agonists could have therapeutic potential to increase TREM2 levels and treat patients with HAND.

INTRODUCTION

HIV associated neurocognitive disorders (HAND) affect up to 50% of people living with HIV and encompass a range of symptoms including mood, motor and cognitive alterations. Microglia play an important role in neuroinflammation and exist on a spectrum of phenotypes ranging from proinflammatory and toxic, M1 to anti-inflammatory and protective, M2. In this study, we induced the M1 phenotype with HRS, GP120, TAT, tenofovir alafenamide fumarate (TAF), A β and interleukin-1 β (IL-1 β). We induced the M2 phenotype with cannabinoid receptor agonists, WIN 55, 212-2 (WIN) and tetrahydrocannabinol (THC).

STUDY DESIGN

HMC3 cells were used as a model for microglia. Cells were cultured in 10cm dishes until they reached confluency. We split cells into 12 well plates and treated with HRS for 24 hours prior to extracting RNA for RT-qPCR. In addition, cells were split into 12 well plates for treatment with siRNA against TREM2 and cannabinoid receptor agonists, WIN and THC, followed by protein and RNA extraction for western blot and RT-qPCR, respectively. Lastly, cells were split into 96 well plates and incubated in fluorescent amyloid beta and analyzed with a fluorescent imager.



RESULTS

HIV Relevant Stimuli: Cytokine and TREM2 RT-qPCR

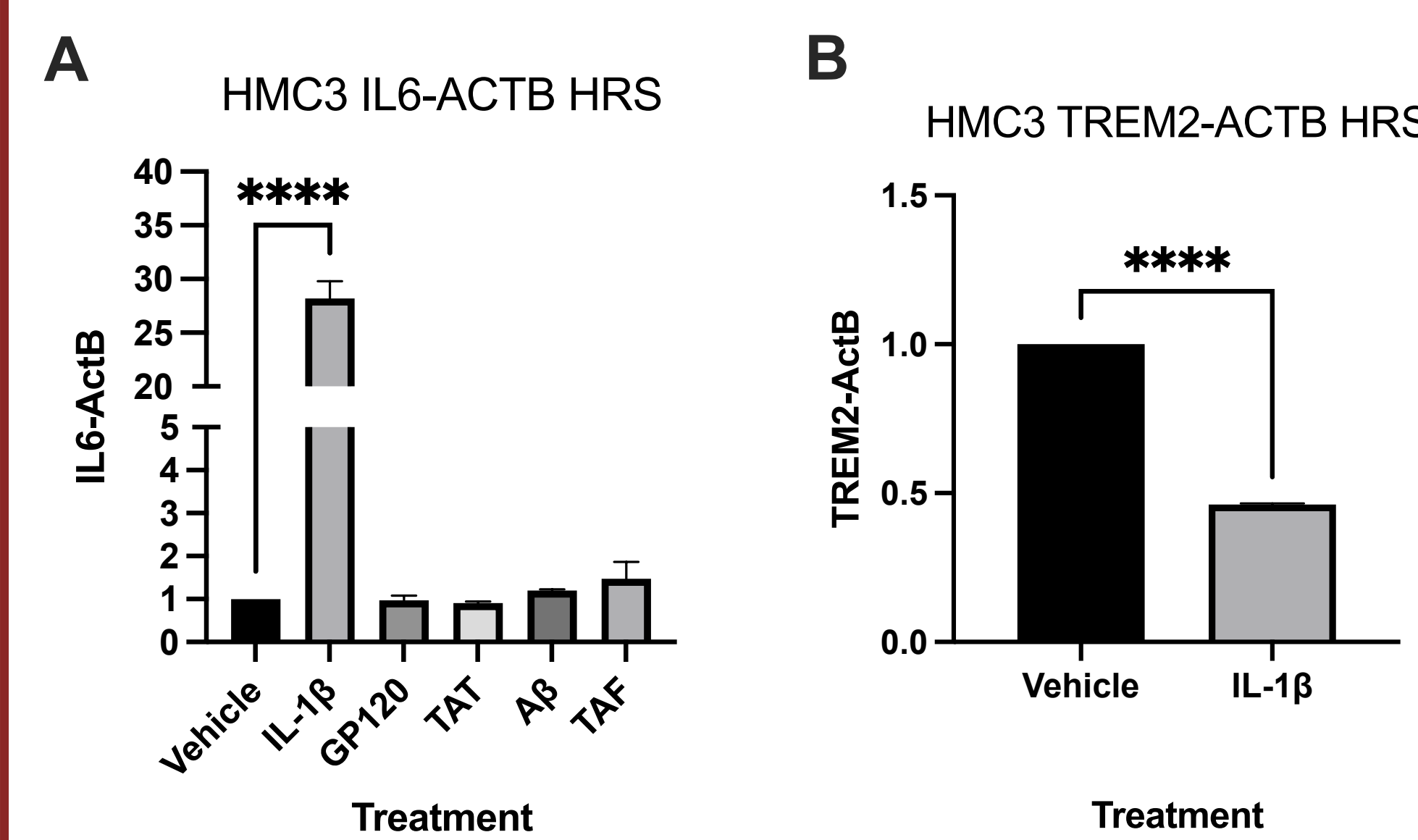


Figure 1: HRS increases production of inflammatory cytokines in HMC3 cells and decreases TREM2. Treatment with IL-1 β significantly increased production of IL-6 (1A). TAF treatment increased IL-6 levels as well, but this did not reach significance (1A). IL-1 β significantly decreased TREM2 levels (1B).

TREM2 Knockdown A β Phagocytosis Assay

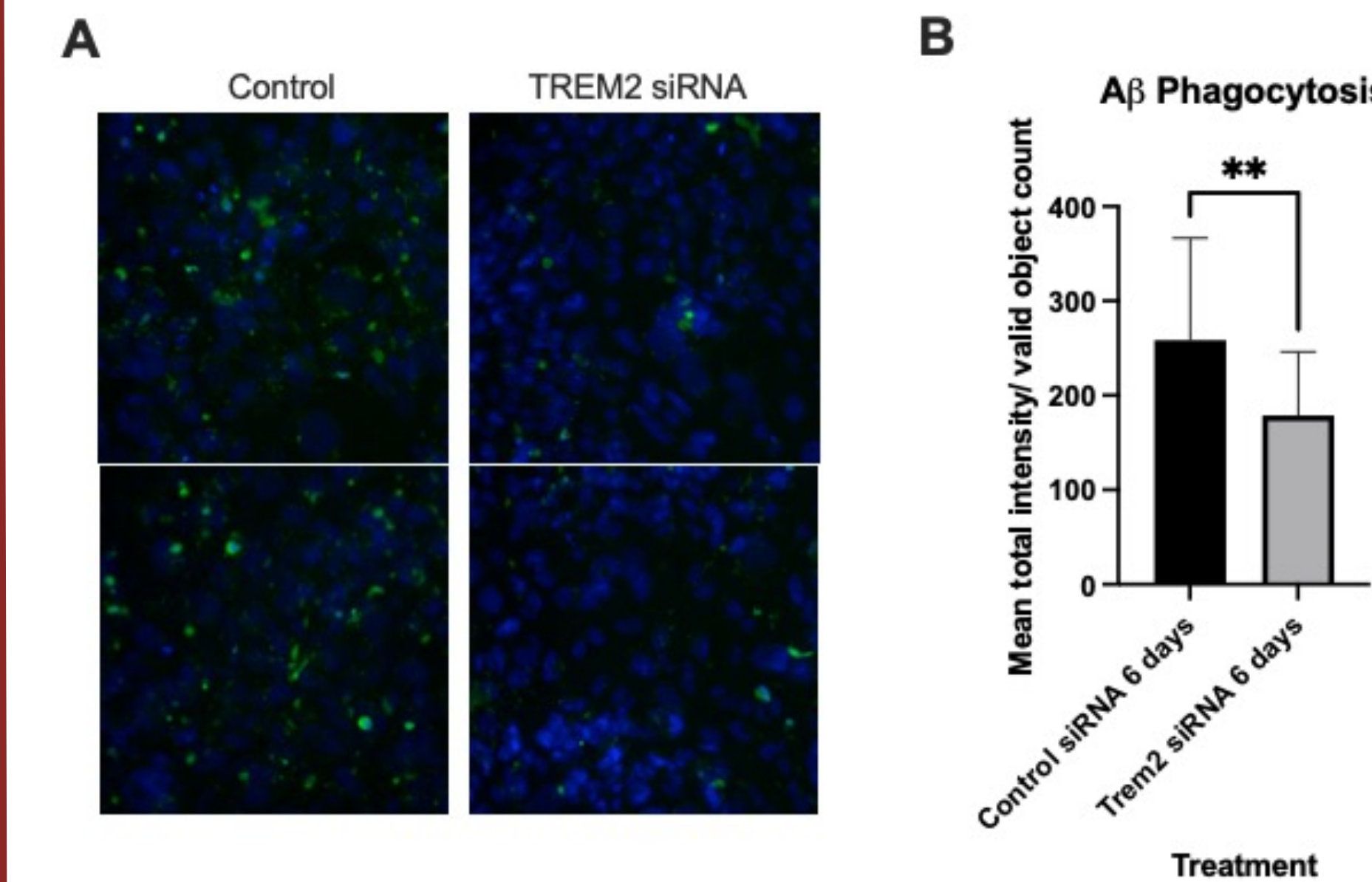


Figure 3: TREM2 knockdown reduces HMC3 phagocytosis of A β . A β fluorescence (green) can be seen at an increased intensity in vehicle treated cells compared to cells incubated with TREM2 siRNA (3A). Quantification of fluorescence per cell revealed a significant decrease in A β phagocytosis in TREM2 knockdown cells compared to controls p=0.0082 (3B).

TREM2 siRNA Western Blot

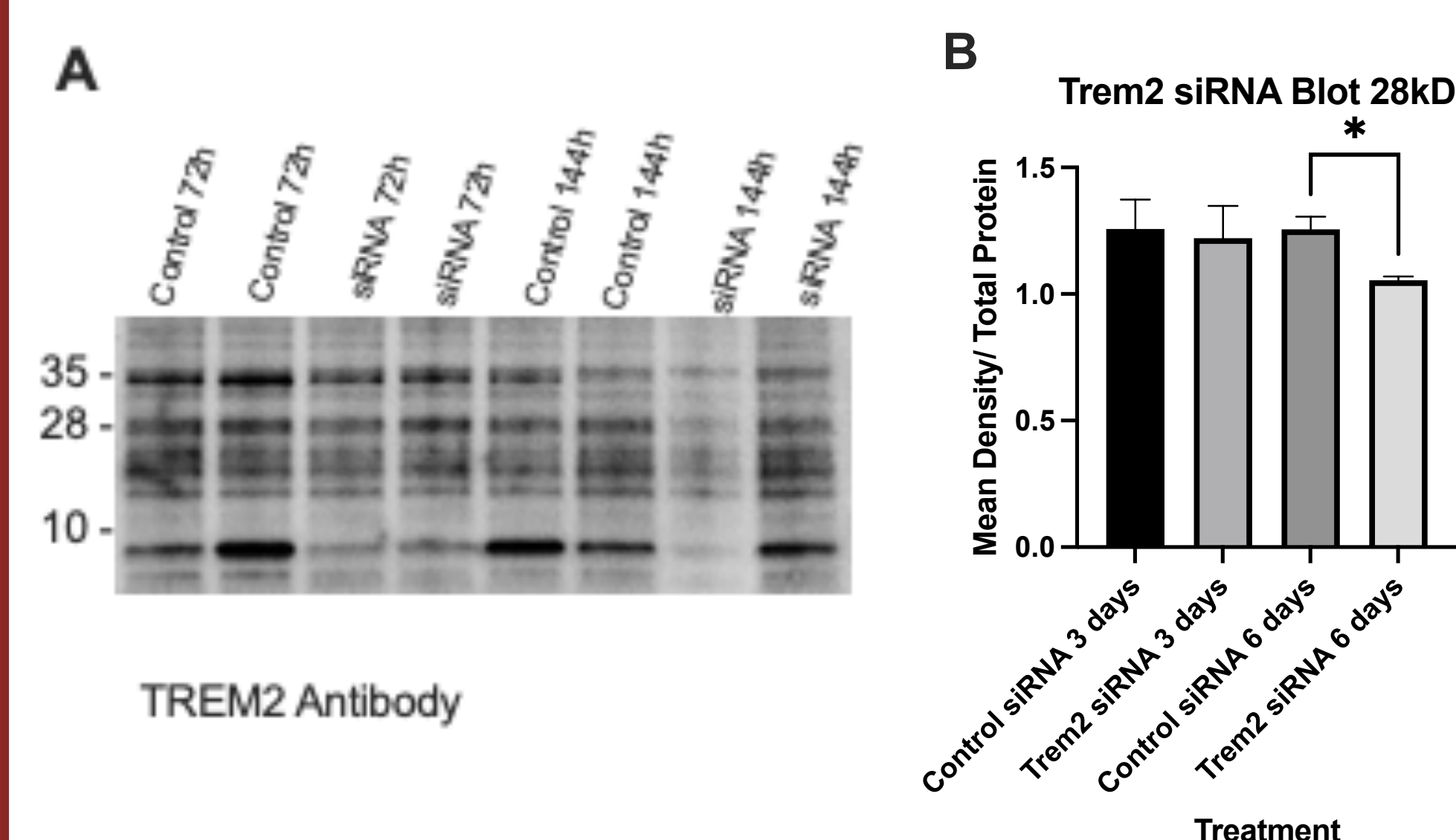


Figure 2: siRNA TREM2 knockdown in HMC3 cells. Bands for TREM2 at 35, 28, and 10 are reduced after 144hours of incubation with siRNA (2A). These bands were compared to total protein transferred. Quantification of the 28kDa band showed significant decrease in TREM2 after 144hours of incubation in siRNA (2B).

Cannabinoid Receptor Agonist TREM2 and Cytokine RT-qPCR

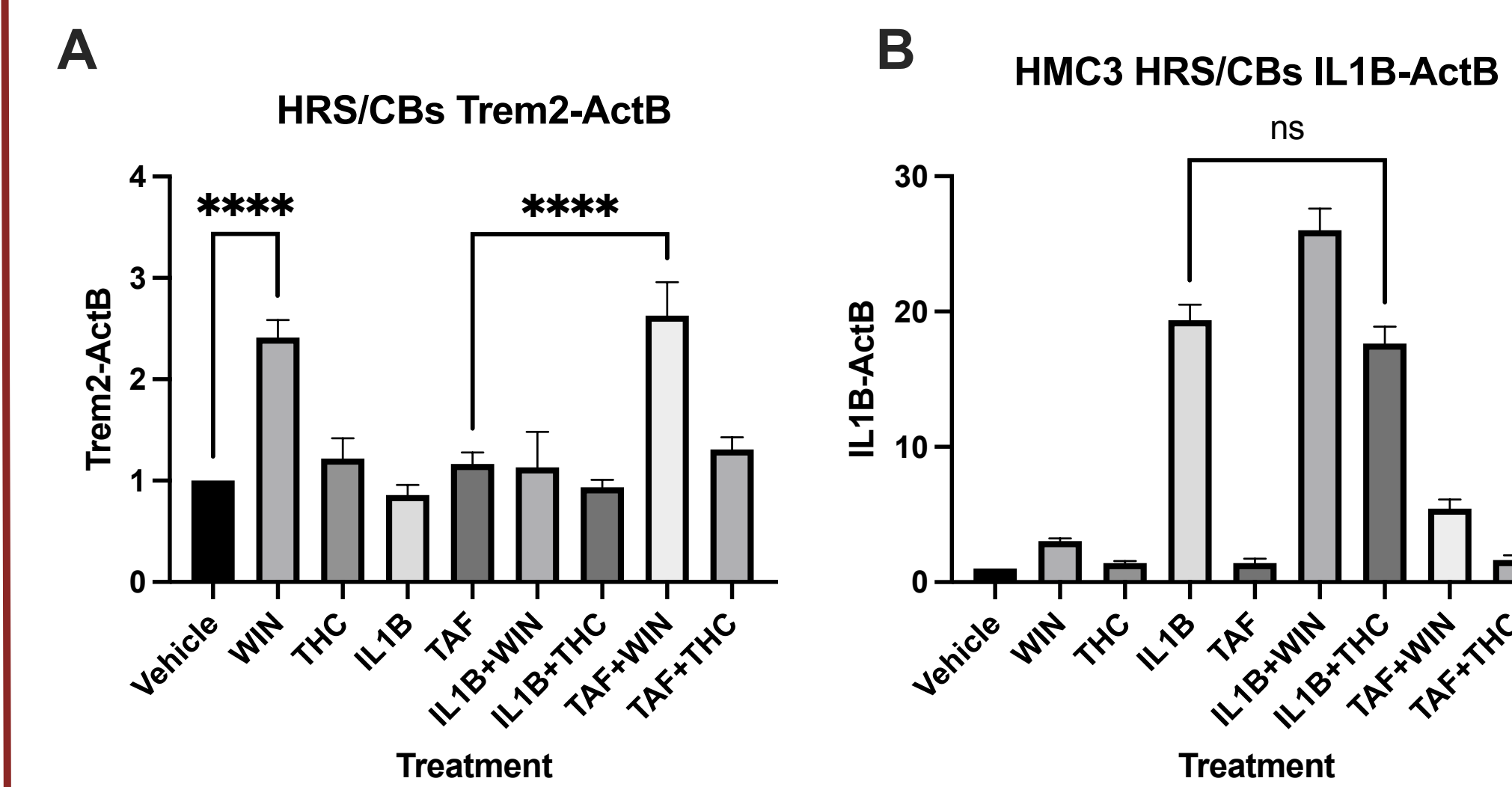


Figure 4: Cannabinoid receptor agonists and HRS alter TREM2 and cytokine production in HMC3 cells. Treatment with WIN increased TREM2 production in HMC3 cells alone and in combination with TAF (4A). Treatment with IL-1 β and IL-1 β + WIN significantly increased production of IL-1 β (4B). Treatment with THC slightly reduced IL-1 β production compared to IL-1 β treatment alone, but this was not significant (4B).

Cannabinoid Receptor Agonist Western Blot for TREM2

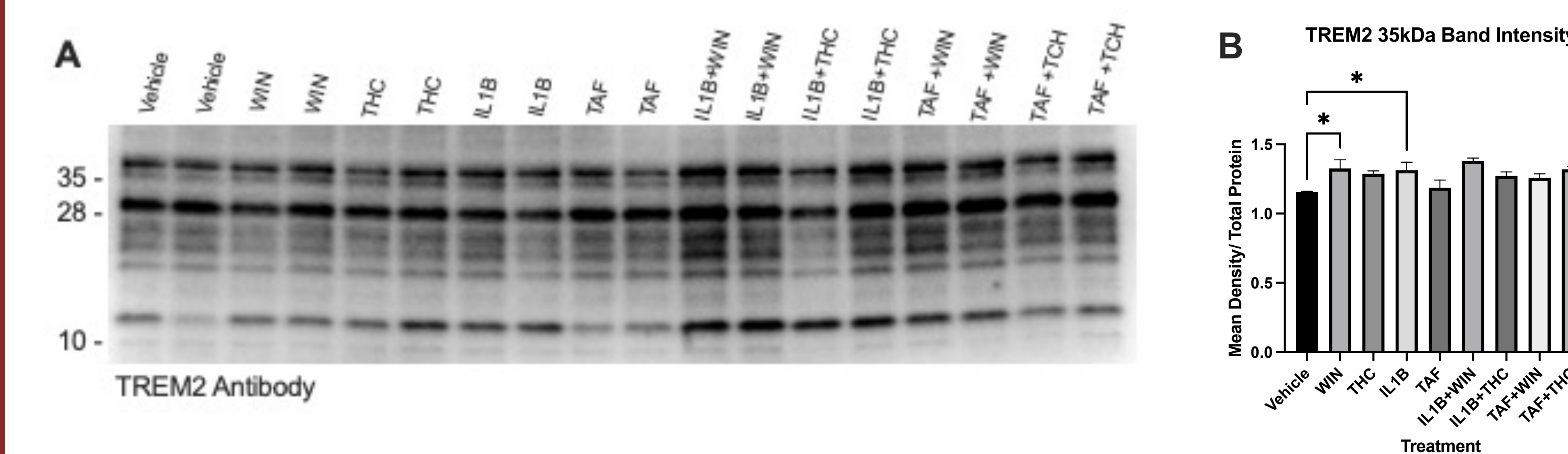


Figure 5: Cannabinoid receptor agonists and HRS alter TREM2 expression levels. The 35, 28, and 10kDa bands for TREM2 can be seen altered in treatment groups including WIN, THC, IL-1 β , and TAF (5A). These bands were compared to total protein transferred to the blot. Quantification of the 35kDa band revealed increased TREM2 in WIN and IL-1 β treated cells (5B). The 10kDa band showed an increase in TREM2 in both THC and IL-1 β treated groups (graph not shown).

DISCUSSION

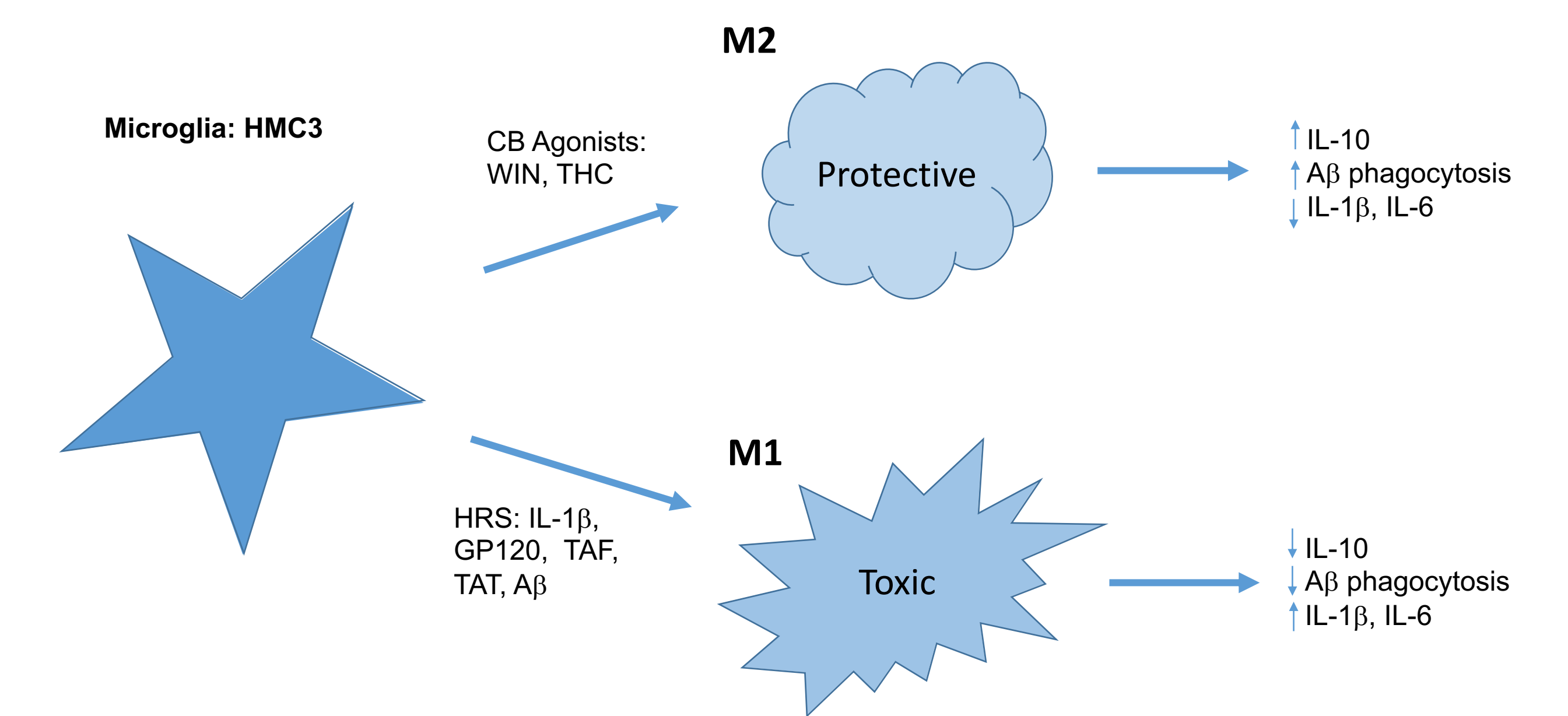


Diagram 1: Microglia are classified as either anti-inflammatory and neuroprotective, M2, or inflammatory and neurotoxic, M1. The M1 phenotype can be induced by HRS, and the M2 phenotype can be induced by cannabinoid receptor agonists. M2 is associated with anti-inflammatory cytokines, increased TREM2, and increased A β phagocytosis. M1 is associated with increased inflammatory cytokines, decreased TREM2, and decreased A β phagocytosis.

Our results show that the toxic M1 microglial phenotype is upregulated with HRS and that this is associated with decreased levels of TREM2. This data supports previous findings that decreased membrane bound TREM2 levels and increased soluble TREM2 were associated with increased neuroinflammation and A β in HIV+ brains (Fields JA, 2018). Our finding of increased TREM2 in IL-1 β treated cells on western blot could be due to an increase in the soluble TREM2 which was not measured in this study. In addition, our results finding a protective value of cannabinoid receptor agonists supports our previous findings that WIN reduces proinflammatory gene expression induced by IL-1 β in astrocytes (Fields JA, 2020). Additional studies are needed to fully understand the role of TREM2 in HIV induced alterations of microglia phenotype and the therapeutic potential of cannabinoid receptor agonists. In the future, we plan to repeat these experiments with a longer exposure to HRS and in an additional model with peripheral blood monocyte derived macrophages. We also plan to use a plasmid overexpressing TREM2 to further isolate the role of this protein.

CONCLUSION

TREM2 is an important regulator in the phenotypic expression of HMC3 cells. When HMC3 cells were treated with HRS, inflammatory cytokine expression was increased and TREM2 levels were decreased (Figure 1). These findings indicated that HIV relevant stimuli induces the proinflammatory and toxic M1 microglial phenotype and that this is associated with a decreased amount of TREM2. In addition, TREM2 knockdown at the protein level (Figure 2) significantly decreased the ability of the HMC3 cells to phagocytose A β (Figure 3). This indicates that TREM2 is essential for maintaining the protective, M2, phenotype of microglia and that decreased levels will induce the M1 phenotype. Treatment with cannabinoid receptor agonists significantly increased the levels of TREM2 at the RNA and protein level (Figure 4 and 5). However, WIN treatment did not decrease the expression of the proinflammatory cytokine, IL-1 β . THC treatment did decrease the levels of IL-1 β , but this was not significant. Additional experiments are necessary to elucidate the therapeutic potential of cannabinoid agonists on microglial phenotypic expression.

ACKNOWLEDGEMENTS

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