The Role of CD33 in Microglial Neuroinflammation and Phagocytosis

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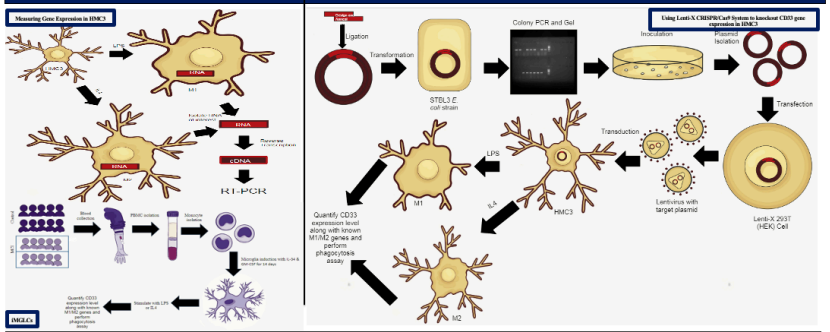
Introduction

Alzheimer's disease (AD) is the leading cause of dementia worldwide, where patients often experience memory loss and the inability to perform daily tasks.[1] β-amyloid (Aβ) aggregation, tau-proteins tangles, and neuroinflammation are the main features of AD. Microglia are the primary immune cells in the brain that remove the extra accumulation of Aβ or tau-proteins via phagocytosis.[2] Genetic studies indicate that risk genes for AD are highly expressed in microglia. Further evidence shows that patients with AD have higher expression of CD33, a microglial transmembrane receptor.[3] However, the functions of those risk genes are largely unknown during AD development.

In this study, we plan to manipulate CD33 expression in microglia and examine microglial functions with different CD33 expression levels. We will first check the CD33 expression patterns during microglial pro-inflammatory (M1) or anti-inflammatory (M2) activation. For this purpose, we will stimulate a human microglial cell line (HMC3) with lipopolysaccharide (LPS) into the M1 phenotype and with interleukin (IL)-4 into the M2 phenotype. Gene expression of CD33 along other well-known M1/M2 genes will be quantified by real-time-polymerase chain reaction (RT-PCR). The phagocytic capacities under each activation will also be measured with fibril Aβ phagocytosis. Next, we will use the CRISPR-Cas9 system to knock out CD33 gene expression in HMC3 and study the phagocytic capacities of the transduced cells. Preliminary data suggests that higher CD33 expression is a consistent phenotype seen in AD patients. Along with using HMC3, we will study the functions of CD33 in a microglia-like cellular model (iMGLCs) induced from human peripheral blood monocytes. Studying the role of CD33 in AD development will facilitate novel therapeutic targets for AD treatment.

Materials and Methods



Background Information

CD33: Participates in downregulating cell proliferation, in promoting growth in immune cells, in blocking the release of certain cytokines, in modulating cell apoptosis, and in regulating phagocytosis. Its role in M1 or M2 microglial responses is not completely clear.[4]

TNF-α: Regulates cell apoptosis, differentiation, growth, metabolism, and other biological pathways; induces M1 microglia to release pro-inflammatory cytokines.[5]

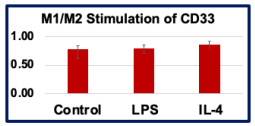
TGF-β1: Involved in the process of cell growth, survival, and differentiation; thought to be involved in anti-inflammatory responses.[6]

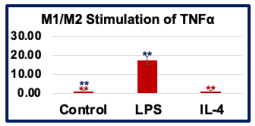
ABCA1: Regulates transport systems in and out of the cells; involved in anti-inflammatory responses in M2 microglia.[7]

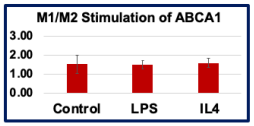
Arginase 1 (Arg-1): Participates in cell healing as well as cell restructuring; participates in anti-inflammatory responses in M2 microglia.[8]

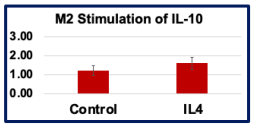
IL-10: Regulates the development of antibodies and cell growth; suppresses inflammatory responses in M2 microglia.[9]

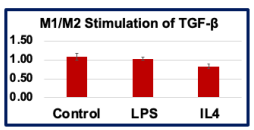
Results

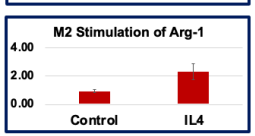


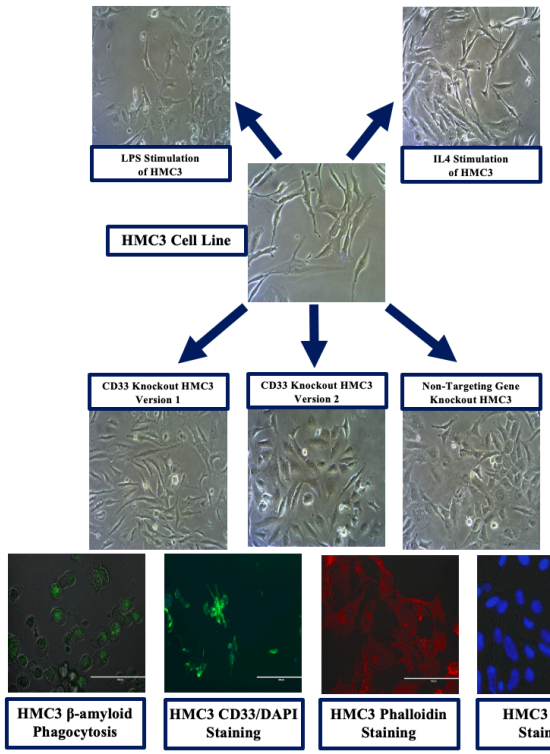


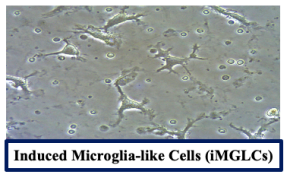


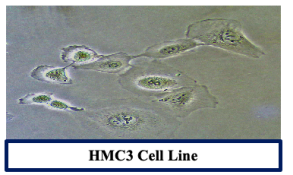


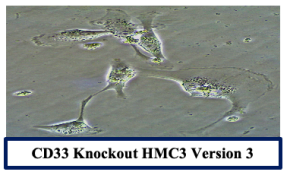












Conclusion

Our study focused on the role of CD33 in human microglial inflammatory and phagocytic responses. Using LPS to stimulate the cells into the M1 pro-inflammatory state and IL4 to stimulate the cells into the M2 anti-inflammatory state, we quantified the expression levels of our target gene CD33 and other known M1/M2 genes. We found no significant upregulation in the expression of CD33 in M1 or M2 stimulated HMC3. As a control, TNF-α, a known pro-inflammatory gene, was significantly upregulated in LPS-stimulated HMC3. IL-10, a known anti-inflammatory gene, was also expressed at higher levels in IL4-stimulated HMC3. We used the Lenti-X CRISPR/Cas9 system to knockout CD33 in HMC3 as well to study its effects on microglial inflammation and phagocytosis. However, the transduced cells in our first attempt at knocking out CD33 did not reach full confluency for us to study them. Furthermore, we compared differences between HMC3 and a human induced microglia-like cellular model (iMGLCs). We found that the two types of human microglia shared similar phagocytic abilities in the presence of β-amyloid. We observed that the two types of cells responded similarly to LPS as well. Unlike the immortalized HMC3, the iMGLCs were more sensitive to changes and grew at lower densities.

Future Directions

We will continue to study the functions of CD33 in human microglia and dissect pathways associated with the AD risk gene. Our first attempt at knocking out CD33 in HMC3 was not successful because the cell line exhibited poor cell growth after transduction. However, we recently purchased new ATCC HMC3 and have already begun our second attempt at knocking out CD33. Our experience with the new ATCC HMC3 has shown fast and positive cell growth and response. After the newly transduced HMC3 reach full confluency, we will stabilize the cell line and examine the inflammatory and phagocytic responses of the CD33 knockout microglia. We also plan to standardize and stabilize our iMGLCs model to study the role of CD33 in those human cells. We will compare CD33's functions in the immortalized HMC3 cell line and in the iMGLCs model.

References

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Acknowledgement

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