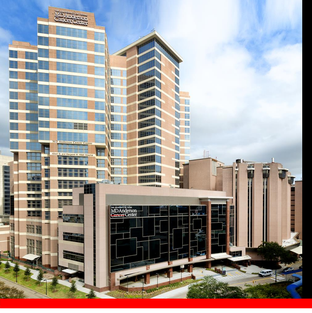


Characterization of Mouse Ms4a4a and Ms4a6d Antibodies

Isabella Rodriguez[1], Ronnie T. Trinh[2], Yaima Luzardo Lightfoot, PhD[2], William J. Ray, PhD[2]

[1]Neurodegeneration Consortium, The University of Texas MD Anderson Cancer Center, Houston, Texas

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Making Cancer History®

Introduction

MS4A variants genetically associate with Late Onset Alzheimer's Disease (LOAD).

MS4A4A and MS4A6A are exclusively expressed by microglia in the brain.

Microglia have been shown to play a critical role in AD pathogenesis, with several AD-associated risk genes being microglial.

In addition to the MS4A proteins, TREM2 is a genetically validated AD target and associations between MS4A4A and TREM2 have been previously noted.

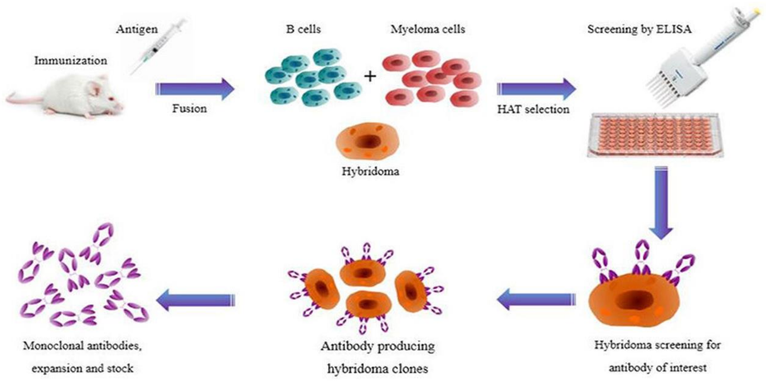
Tools to investigate the pathophysiological role of MS4A4A and MS4A6A in AD are lacking, and targeting these proteins with small-molecule drugs has proven difficult.

Our overarching goal is to develop functional antibodies targeting MS4A4A and MS4A6A to induce a protective microglial phenotype in humans.

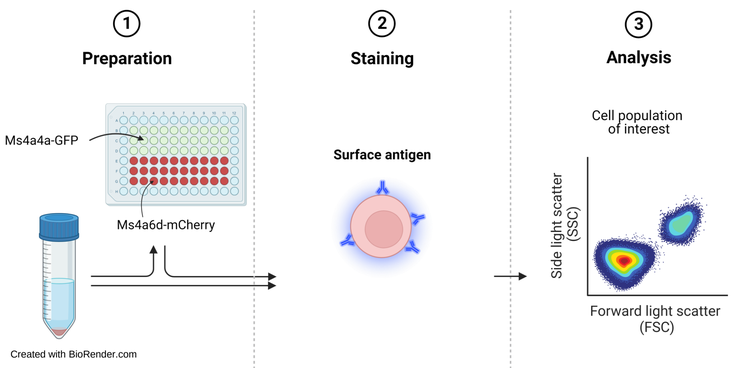
The aim of this study is to develop mouse-specific antibodies, which can then be used to obtain in vivo data before progressing the human therapeutics into the clinic.

Summary of Methods

Antibody Development & Primary Screen: Mice were immunized with peptides generated from the large extracellular loop of Ms4a4a or Ms4a6a. Serum titers were measured by peptide-specific ELISAs and hybridomas were generated using spleen B cells from mice showing appropriate immune reactivity. Hybridoma supernatants were used to screen for specific clones recognizing Ms4a4a or Ms4a6d.



Secondary Screen: As ELISAs only show binding to linear peptide, hybridoma supernatants were then screened for cell surface binding by flow cytometry using cell lines overexpressing either Ms4a4a or MS4a6d. Clones recognizing the native protein were selected for antibody production.



In Vitro Testing on BMDMs (bone marrow-derived macrophages): BMDMs were isolated and treated with purified antibodies to measure soluble TREM2 levels and cell health under supplement-limiting conditions.

Tg 5xFAD Microglia Analysis: Microglia were isolated from control and transgenic 5xFAD mice (AD mouse model). Cells were stained with antibodies to measure levels of Ms4a4a and Ms4a6d in health and disease.

In Vivo Dosing of C57BL/6J Mice: Wild-type mice were dosed once a week (4x) with either Ms4a4a or Ms4a6d antibody. Plasma sTREM2 levels were measured by ELISA. Microglia were isolated for RNA sequencing to measure gene expression changes.

Results

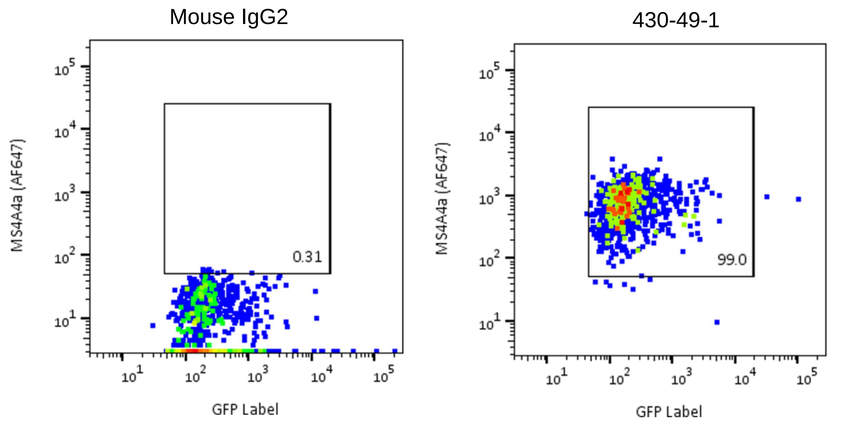


Figure 1: Mouse IgG2 control and Ms4a4a antibody clone 430-49-1 flow cytometry analysis. Purified antibodies from hybridoma clones that showed binding by FACS were obtained from MDA Core and further screened to be used for future analysis. Clone 430-49-1 shows binding to Ms4a4a-GFP-positive cells.

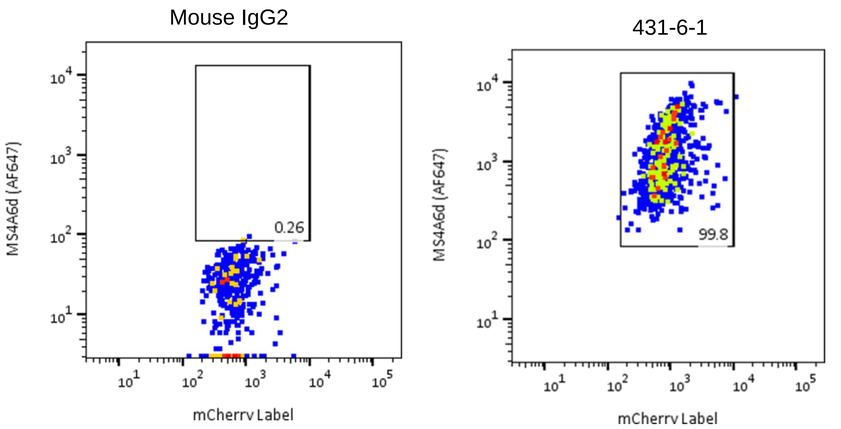


Figure 2: Mouse IgG2 control and Ms4a6d antibody clone 431-6-1 flow cytometry analysis. Purified antibodies from hybridoma clones that showed binding by FACS were obtained from MDA Core and further screened to be used for future analysis. Clone 431-6-1 binds Ms4a6d-mCherry-positive cells.

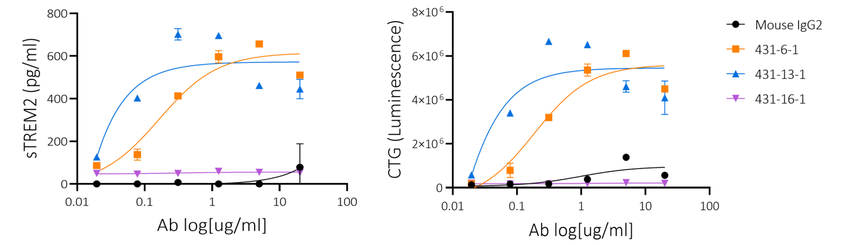


Figure 3: Anti-Ms4a6d-treated BMDMs show increased sTREM2 levels and improved cellular health. Mouse BMDMs were isolated and treated with purified antibodies for 72 hours. BMDMs serve as a cell model for testing mouse Ms4a4a and Ms4a6d antibodies. Soluble TREM2 (sTREM2) levels and ATP content measured by ELISA and CTG assay, respectively.

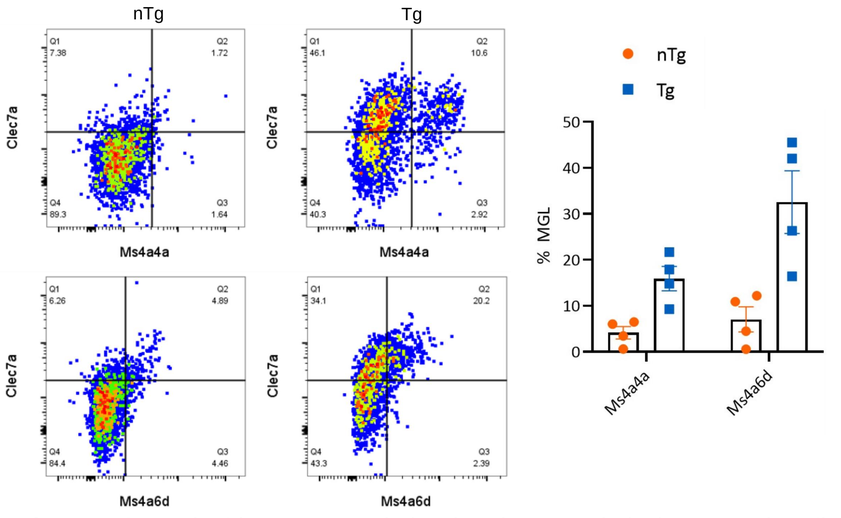


Figure 4: 6-month-old non-transgenic (nTg) and transgenic (Tg) 5xFAD microglia were stained with mouse antibodies. Percent of microglia expressing Ms4a4a or Ms4a6d after staining with purified mouse antibodies in transgenic and non-transgenic mice. Ms4a4a and Ms4a6d expression is increased in microglia of transgenic mice. Clec7a is typically upregulated in clusters of activated microglia.

Results (Continued)

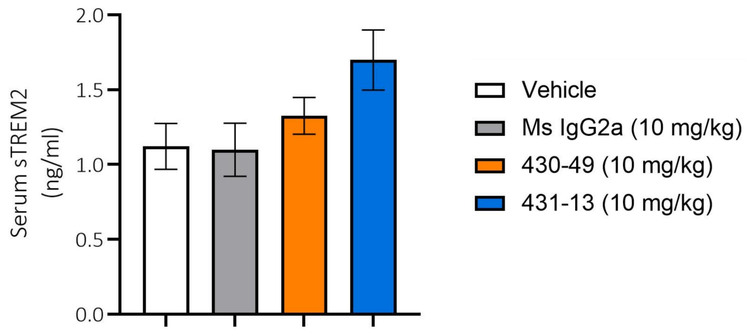
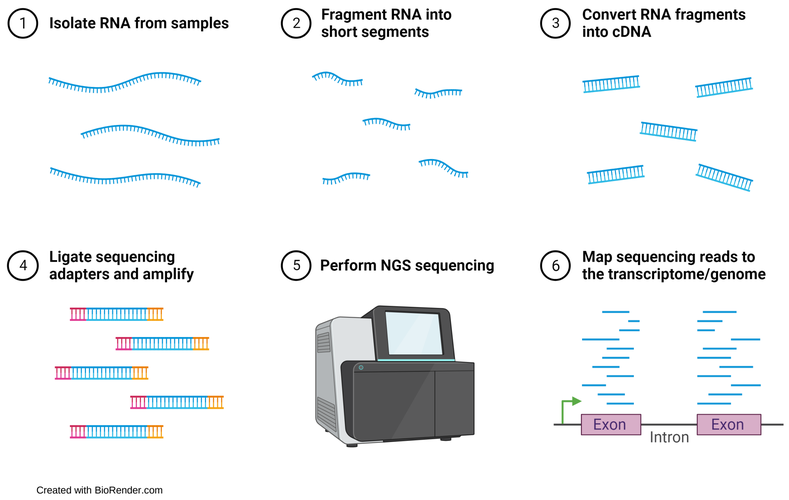


Figure 6: sTREM2 levels in C57BL/6J mouse plasma. Mice were treated with Ms4a4a or Ms4a6d antibodies once a week for four weeks. Levels of sTREM2 were increased in mice dosed with these antibodies.

Conclusions

- Antibodies targeting Ms4a4a and Ms4a6d specifically bind the proteins of interest and show functional effects in vitro
- Further in vivo characterization is required, including increasing concentrations of antibodies to ensure sufficient brain exposure
- Future directions: Data are pending from RNA-seq analysis of microglia isolated from mice dosed with antibodies to investigate gene expression changes in this cell population after antibody dosing



Acknowledgements

I am grateful to MD Anderson's Summer Undergraduate Research Program (SURP) for allowing my participation in the Neurodegeneration Consortium's AD research.

I also would like to acknowledge my mentors and the NDC funding provided by the Belfer Family Foundation, the Oskar Fischer Project, and the Bowes Family Foundation

References

1. Mattiola I, Mantovani A, Locati M. The tetraspan MS4A family in homeostasis, immunity, and disease. Trends Immunol. 2021 Sep;42(9):764-781. doi: 10.1016/j.it.2021.07.002. Epub 2021 Aug 9. PMID: 34384709.
2. Deming Y, Filipello F, Cignarella F, Cantoni C, Hsu S, Mikesell R, Li Z, Del-Aguila JL, Dube U, Farias FG, Bradley J, Budde J, Ibanez L, Fernandez MV, Blennow K, Zetterberg H, Heslegrave A, Johansson PM, Svensson J, Nellgård B, Lleo A, Alcolea D, Clarimon J, Rami L, Molinuevo JL, Suárez-Calvet M, Morenas-Rodríguez E, Kleinberger G, Ewers M, Harari O, Haass C, Brett TJ, Benitez BA, Karch CM, Piccio L, Cruchaga C. The *MS4A* gene cluster is a key modulator of soluble TREM2 and Alzheimer's disease risk. Sci Transl Med. 2019 Aug 14;11(505):eaau2291. doi: 10.1126/scitranslmed.aau2291. PMID: 31413141; PMCID: PMC6697053.
3. Qin Q, Teng Z, Liu C, Li Q, Yin Y, Tang Y. TREM2, microglia, and Alzheimer's disease. Mech Ageing Dev. 2021 Apr;195:111438. doi: 10.1016/j.mad.2021.111438. Epub 2021 Jan 28. PMID: 33516818.
4. Schwabe T, Srinivasan K, Rhinn H. Shifting paradigms: The central role of microglia in Alzheimer's disease. Neurobiol Dis. 2020 Sep;143:104962. doi: 10.1016/j.nbd.2020.104962. Epub 2020 Jun 12. PMID: 32535152.
5. Shiferaw, Haimi. "Hybridoma Technology: Production of Monoclonal Antibodies." *IBT BIOSERVICES*, https://www.ibtbioservices.com/assays-and-protein-expression/production-of-monoclonal-antibodies/. Accessed 1 Aug. 2023.