Development of novel rabbit monoclonal antibodies to characterize microglial activation states in murine models of Alzheimer's disease

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INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common form of dementia worldwide. Neuroinflammation is an important feature of AD pathology, but the precise contribution of neuroinflammation on disease progression is poorly understood. Microglia, the brain's resident macrophages, are likely to play an important role in initiating and maintaining neuroinflammatory responses that contribute directly or indirectly to AD etiology.

Several genome-wide association studies in human AD patients identified genes enriched or uniquely expressed in microglia. Moreover, single-cell RNA sequencing (scRNA-seq) studies identified multiple microglia-enriched genes that are upregulated in the context of disease, both in human AD tissue as well as mouse models of AD. Development of tools to these specific genes or gene products can be used to identify disease-associated microglial states and further our understanding of the specific neuroinflammatory responses that contribute to disease.

Here we have developed and validated a cohort of rabbit monoclonal antibodies that can be used to detect these microglial gene products. We used multiplexing techniques to establish microglial enrichment of these targets in mouse models of AD. Within this cohort, we highlight Cathepsin D, a lysosomal aspartyl protease involved in protein degradation that is enriched in microglia, particularly in the context of disease. Here we showcase Cathepsin D expression in both wild-type and a mouse model of AD, focusing attention on the co-localization of Cathepsin D with Iba1 positive microglia.

We continue to develop a comprehensive portfolio of monoclonal antibodies to further characterize microglia cellular processes and activation states to understand the role of microglia in neurodegenerative diseases.

Disease-Associated Microglia (DAM)

General	Homeostatic	Stage 1 DAM	Stage 2 DAM
Iba1 CD11b	TMEM119	Cathepsin D CD68 ASC/TMS1	GPNMB Ship1 Galectin-3

Diagram 1:
Disease associated microglia (DAM) transition from a homeostatic state to stage 1 and stage 2 (TREM2-dependent) DAM [1]. Key targets listed according to their role in each microglial stage.

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METHODS

- Recombinant monoclonal antibodies against identified genes enriched or uniquely expressed in microglia were analyzed on a mouse model of AD by immunofluorescence utilizing CST's Immunofluorescence Protocol. Samples were multiplexed using a combination of direct and indirect detection and a sequential labeling strategy (Diagram 2).
- Images captured by widefield, high-resolution imaging using a Leica SP8 confocal microscope.

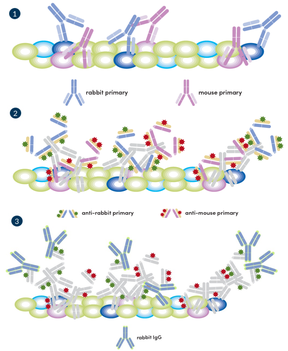


Diagram 2:
Illustrated diagram showcasing key IF protocol steps for direct and indirect detection multiplexing utilizing unconjugated and fluorophore-conjugated antibodies. The image demonstrates incubating first with an unconjugated primary antibody (1), detecting primary antibodies with corresponding fluorophore-conjugated secondaries (2), blocking free secondary binding sites with appropriate host immunoglobulin (3) enabling co-staining with desired fluorophore-conjugated antibodies.

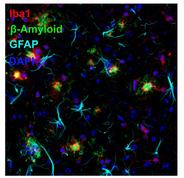
Table 1:

Key Antibodies Used	Catalog #
Iba1/AIF-1 (E4O4W) XP® Rabbit mAb (Alexa Fluor® 647 Conjugate)	78060
Iba1/AIF-1 (E4O4W) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate)	20825
CD11b/ITGAM (E6E1M) Rabbit mAb	17800
TMEM119 (E3W5L) Rabbit mAb	80821
ASC/TMS1 (D2W8U) Rabbit mAb (Mouse Specific)	67824
CD68 (E3O7V) Rabbit mAb	97778
SHIP1 (E8M5D) Rabbit mAb	79877
GPNMB (E7U1Z) Rabbit mAb	90205
Galectin-3/LGALS3 (E7B6R) Rabbit mAb	89572
Cathepsin D (E7Z4L) XP® Rabbit mAb	88239

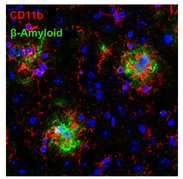
Additional antibody markers can be found at cellsignal.com

Figure 1. Microglial targets imaged on a mouse model of AD

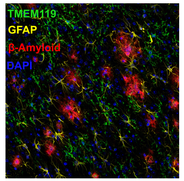
Iba1/AIF-1
Ionized calcium-binding adaptor molecule 1 (Iba1), also known as allograft inflammatory factor 1 (AIF-1), is a cytoplasmic calcium binding protein uniquely expressed in cells of monocytic lineage and is, therefore, widely used as a marker for microglia/macrophages in the brain [2].



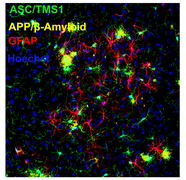
CD11b/ITGAM
Cluster of differentiation molecule 11b (CD11b)/Integrin alpha M (ITGAM) is a transmembrane protein expressed by myeloid lineage cells, including macrophages and microglia [3].



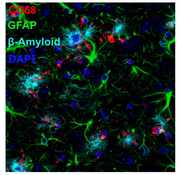
TMEM119
Transmembrane protein 119 (TMEM119) is a cell-surface protein expressed by homeostatic microglia. TMEM119 has been shown to by downregulated in DAM and is an important marker to decipher microglia from infiltrating macrophages in neurodegenerative disease models [4].



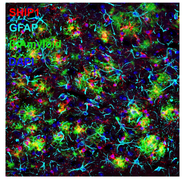
ASC/TMS1
Apoptosis-associated speck-like protein containing a CARD (ASC) has been shown to accumulate in inflammasome-activated macrophages, including microglia, and build up as aggregated called ASC specks [5].



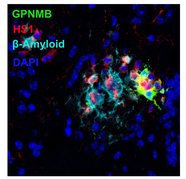
CD68
Cluster of differentiation 68, CD68, is a transmembrane protein widely used as marker of activated phagocytic microglia and macrophages. CD68 has been shown to be upregulated in mouse models of AD [6].



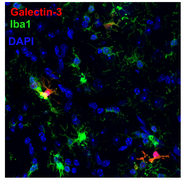
SHIP1
SH2-containing inositol phosphatase, SHIP1, is a hematopoietic phosphatase expressed by microglia that has been associated with late onset Alzheimer's disease. In mouse models of AD, SHIP1 expression increases with disease progression and is expressed by plaque-associated microglia [7].



GPNMB
Glycoprotein nonmetastatic melanoma protein B (GPNMB) has been shown to localize within a distinct population of microglial cells that surround amyloid plaques. In AD mouse models, GPNMB levels increase in an age-dependent manner [8].



Galectin-3
In AD mouse models, it has been shown that microglial galectin-3 expression is induced by Aβ stimulation. Galectin-3 is secreted by microglia where it directly interacts with Aβ, promoting aggregation by decreasing Aβ degradation [9].



Cathepsin D
Cathepsin D is a ubiquitously expressed lysosomal aspartyl protease involved in protein degradation enriched in microglia particularly in the context of disease. It is suggested that Cathepsin D may be involved in the degradation of Aβ [10].

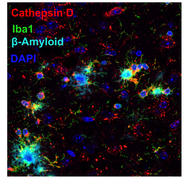


Figure 1: Microglial targets imaged on a mouse model of AD by category
Multiple microglial targets multiplexed and imaged on a mouse model of AD by category. Categories of shown microglial markers include general microglia markers, homeostatic microglia, stage 1 DAM (TREM2 independent), and Stage 2 DAM (TREM2 dependent). Images are labeled by stain color of each antibody used.

General
Homeostatic
Stage 1 DAM
Stage 2 DAM

Figure 2. Cathepsin D is expressed in WT and AD mouse hippocampus

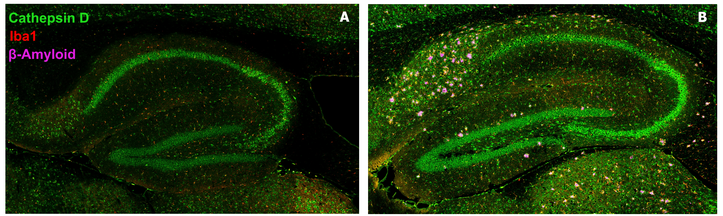


Figure 2: Confocal Tile Scan Analysis of Cathepsin D
Confocal tile scan immunofluorescent analysis of brain from wild-type mouse (A) and an amyloid mouse model of AD (B) using Cathepsin D (green), Iba1/AIF-1 (red), and β-Amyloid (magenta).

Figure 3. Cathepsin D localizes to Iba1+ activated microglia surrounding amyloid plaques in AD mouse model

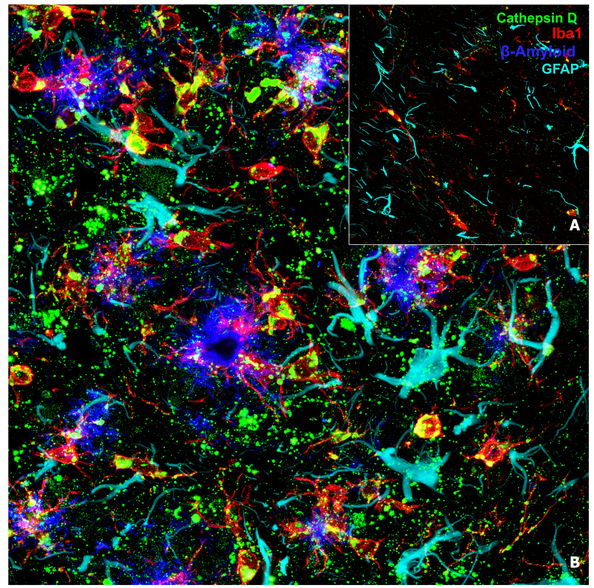


Figure 3: Confocal immunofluorescent analysis of Cathepsin D
Confocal immunofluorescent analysis of brain from a wild-type mouse (A) and an amyloid mouse model of AD (B) using Cathepsin D (green), Iba1/AIF-1 (red), GFAP (cyan), and β-Amyloid (blue).

CONCLUSIONS

- Neuroinflammation is an important feature of AD pathology. Microglia have been implicated in disease through GWAS and scRNA-seq studies.
- We have developed a portfolio of rabbit monoclonal antibodies specific to these targets that have been validated in mouse models of AD.
- We show that Cathepsin D expression is increased in mouse models of AD and co-localizes with Iba1+ microglia surrounding amyloid-beta plaques.
- These antibodies can be used to further characterize microglia cellular processes and activation states to further our understanding of the role microglia play in neurodegenerative diseases.

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