Characterization of a Novel Rat Monoclonal Antibody Against Murine P2RY12 for Specific Detection and Isolation of Microglia

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Introduction

Microglia are the brain and spinal cord-resident macrophages that function as sentinels in maintaining CNS homeostasis. Dysregulation of these sentinels has been associated with neuropsychiatric and neurodegenerative disorders. A major limitation in understanding microglial contribution to cellular processes and their role in disease has been the lack of tools to specifically distinguish these cells from other myeloid cells. In an effort to produce a novel, microglia-specific tool, we have generated a rat monoclonal antibody (clone S16007D) against murine Purinergic Receptor P2Y12 (P2RY12), a highly selective marker for microglial cells that enables immunostaining in histological sections as well as isolation of these cells by Flow Cytometry (FC) and magnetic nanobeads.

Methods

The specificity of the P2RY12 antibody was validated using IHC and FC in murine brain tissue sections and a stable cell line overexpressing murine P2RY12, respectively. Using FC, the phenotype of corresponding tissue resident macrophages was confirmed by their CD45 and CX3CR1 expression in single cell suspensions from mouse brain, spleen, liver, and lungs. In addition, we assessed LPS-induced alterations in P2RY12 expression, an inflammatory stimulus known to downregulate P2RY12 and to induce amoeboid morphology in microglia. We also validated the utility of the P2RY12 antibody to isolate microglia with high purity and yield with BioLegend's MojoSort™ magnetic cell separation system. Single cell suspensions were prepared from C57BL/6 mouse brains using trypsin digestion followed by a 70/37/30% percoll gradient to remove myelin. Microglia were then isolated using biotinylated P2RY12 antibody, followed by incubation with streptavidin nanobeads. Isolated cells were co-stained with CX3CR1 and CD11b as general markers for microglia, and FC quantification demonstrated the purity of microglia above 99%.

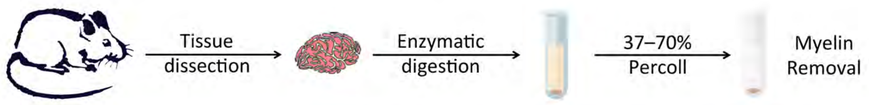
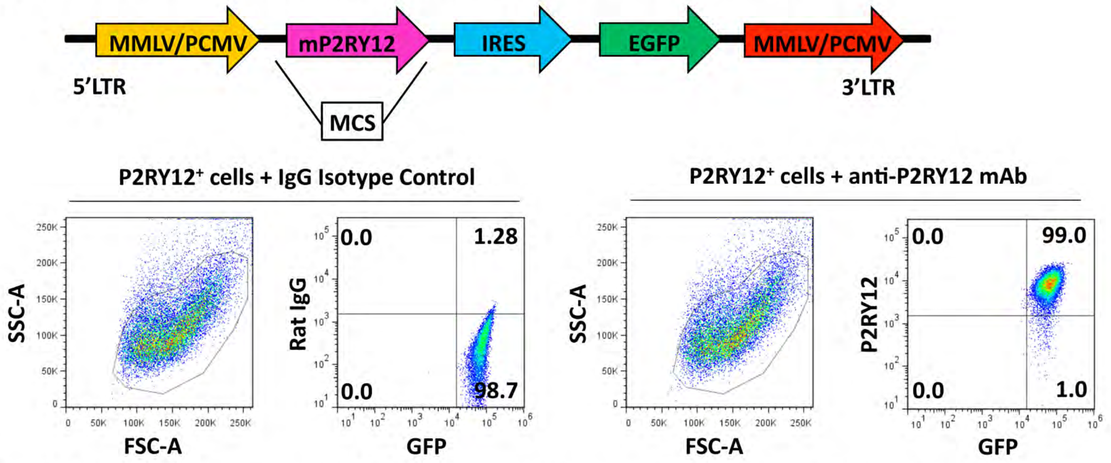
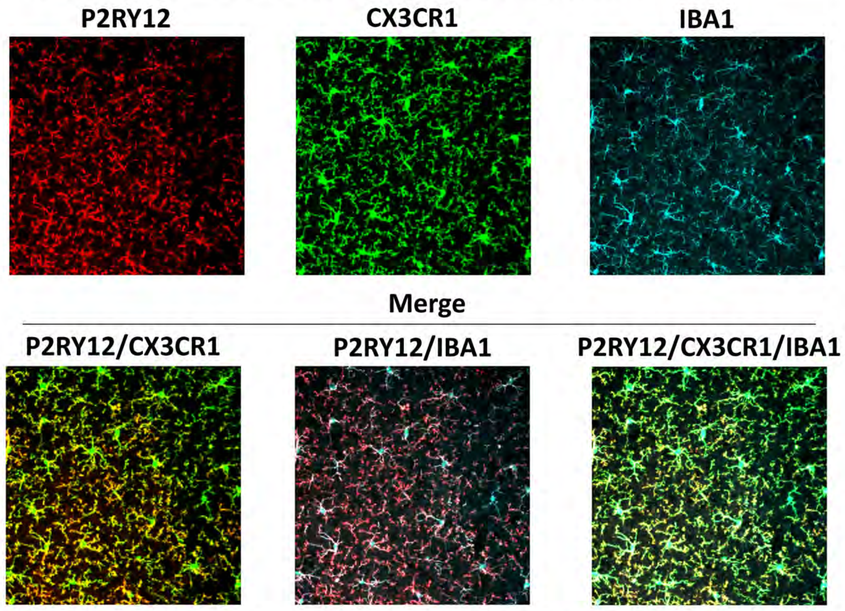


Figure 1: Assessment of murine P2RY12 expression using a P2RY12-IRES-EGFP transfected cell line



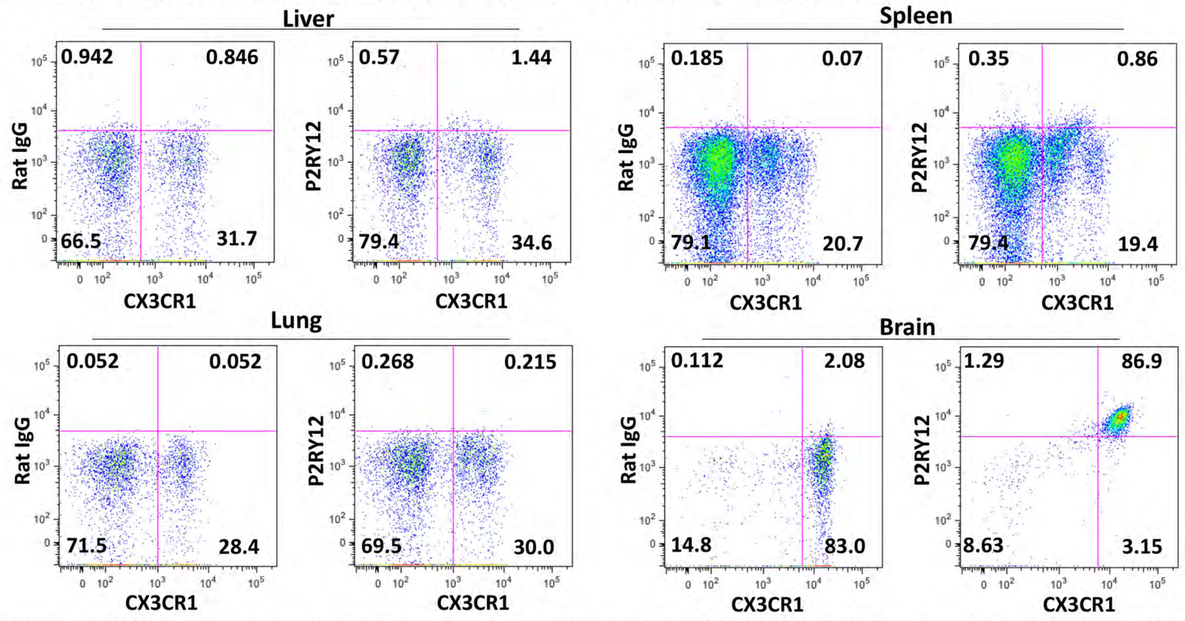
FC analysis of P2RY12 expression in a stable cell line overexpressing murine P2RY12 and GFP. Cells were gated based on size and assessed for P2RY12 and GFP expression.

Figure 2: Co-localization of P2RY12 with CX3CR1 and IBA1 in mouse brain tissue slices



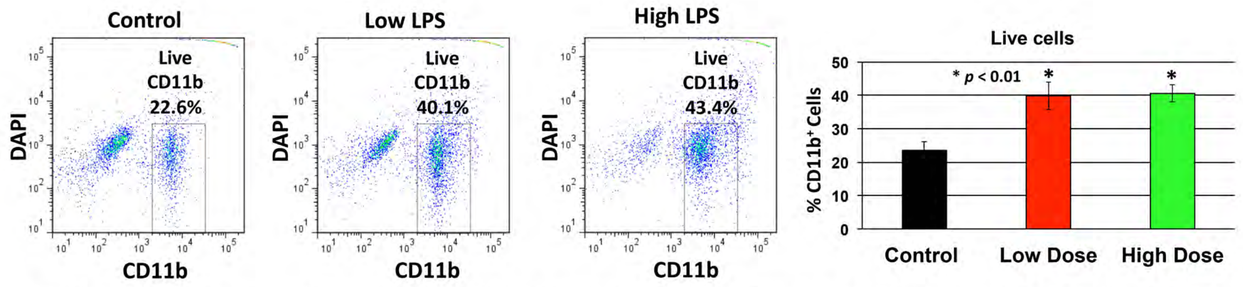
IHC staining on FFPE mouse brain tissue demonstrating the specificity of anti-P2RY12 antibody (clone S16007D) in recognizing microglia. The tissue was co-stained with common microglia markers, anti-CX3CR1 and anti-IBA1 antibodies, to demonstrate co-localization of P2RY12 with these markers in microglial network in the brain.

Figure 3: Expression of P2RY12 in mouse brain but not spleen, liver, or lung assessed by flow cytometry



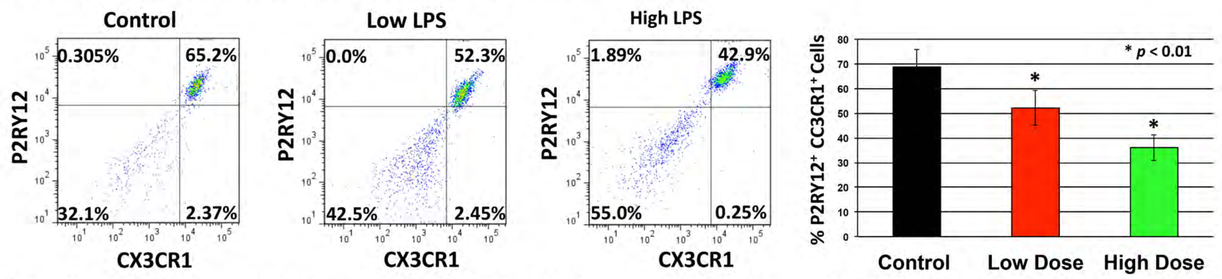
FC analysis of P2RY12 expression in murine tissues demonstrates the specificity of P2RY12 antibody for microglia in the brain. Single cell suspensions were prepared from mouse tissues, gated on CD45 and analyzed for P2RY12 and CX3CR1 expression. These results show high P2RY12 expression in the brain in contrast to negligible expression in peripheral tissues in mice, confirming lack of antibody cross-reactivity with other tissue resident macrophages.

Figure 4: Increased percentage of CD11b$^+$ cells with LPS injection



FC analysis of CD11b expression in control and LPS-injected mice demonstrates an increase in the percentage of CD11b$^+$ cells in mouse brains in response to LPS. Live cells in single cell suspensions were assessed for CD11b expression. Debris/dead cells were gated out using DAPI. On average, low (25 µg) and high (250 µg) dose treatment with LPS resulted in a 2-fold increase in CD11b$^+$ cells in the brain.

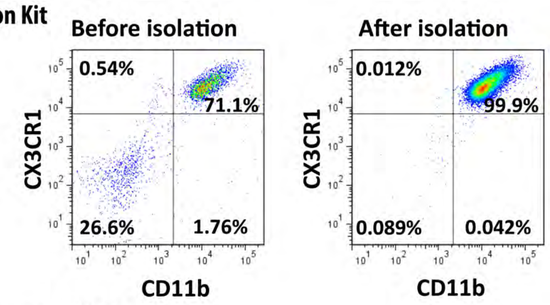
Figure 5: Decreased number of P2RY12$^+$ cells with LPS injection



FC analysis of P2RY12 expression in control and LPS-injected mice shows downregulation of P2RY12 in mouse brains in response to LPS. CD11b$^+$ cells isolated from mouse brains were analyzed for P2RY12 and CX3CR1 expression. Compared to control untreated mice, low (25 µg) and high (250 µg) dose LPS treatments led to 24% and 48% reduction in P2RY12 expression.

Figure 6: Isolation of Microglia with the MojoSort™ Mouse P2RY12 Selection Kit

P2RY12$^+$ microglia were positively isolated using MojoSort™ Selection Kit by incubating single microglial cell suspension with biotin anti-P2RY12 and MojoSort™ Streptavidin Nanobeads, followed by purification using a MojoSort™ Magnet. Debris/dead cells were gated out using 7AAD viability dye. Cells were analyzed for CD11b and CX3CR1 expression.



Conclusions

Here we demonstrate the specificity, versatility, and utility of a novel rat anti-mouse P2RY12 antibody:

- Confirmed utility for IHC, FC, and cell isolation
- Showed antibody detection in a biological context
- Demonstrated antibody utility in isolation of microglial cells

04-0094-00