

Development of a novel microglial specific marker antibody: Anti-P2RY12 guinea pig polyclonal antibody

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Anti P2RY12, Guinea Pig

Abstract

Microglia are the primary resident immune cells in the brain parenchyma and play a variety of roles, such as releasing cytokines and phagocytosis of foreign and dead cells. The heterogeneity of microglial populations and the concept of disease-associated microglia have been proposed, and their relationship to neurological diseases has been actively investigated. On the other hand, macrophages have recently been reported to be resident in specific areas of the central nervous system (CNS). These macrophages, called CNS-associated macrophages (CAMs), are found in the meninges and perivascular spaces, and their properties are very similar to those of microglia. To understand the true microglial dynamics and how they are causally related to CNS disease, it is critical to accurately distinguish microglia from macrophages.

Purinergic receptor P2Y, G-protein coupled 12 (P2RY12) and Transmembrane Protein 119 (TMEM119) are currently used as markers to discriminate between microglia and macrophages. However, commercially available microglial specific marker antibodies are confined to a limited number of animal species and lack versatility in multiple staining. In this study, we set out to develop a guinea pig derived anti-P2RY12 antibody with high versatility, specificity and stable performance in immunohistochemistry.

Materials and methods

Production of anti-P2RY12 guinea pig polyclonal antibodies

1. Immunization

We determined the target sequence from protein conformation prediction by using AlphaFold2 and synthesized peptides corresponding to the C-terminus of P2RY12.

2. Purification

Antiserum was collected from the guinea pigs and purified using protein A affinity chromatography.

Immunohistochemistry

1. Sample

Adult male wild-type mouse (ICR) brain frozen section fixed by 4% PFA. (30 μm)

2. Blocking

0.3% Triton X-100/PBS containing 5% normal goat serum or 3% BSA

3. 1st antibody

P2RY12 (Guinea pig IgG; FUJIFILM Wako; #011-28873; dilution 1:1,000)

Iba1 (Rabbit IgG; FUJIFILM Wako; #019-19741; dilution 1:1,000)

Iba1 (Goat IgG; FUJIFILM Wako; #011-27991; dilution 1:1,000)

TMEM119 (Rabbit IgG; Abcam; #ab209064; dilution 1:100)

Laminin (Rat IgG; Miyata Lab; #HS-2011; dilution 1:200)

4. 2nd antibody

Alexa488-, Alexa594-, or Alexa647-conjugated secondary antibody (Jackson ImmunoResearch; dilution 1:500)

Conclusion

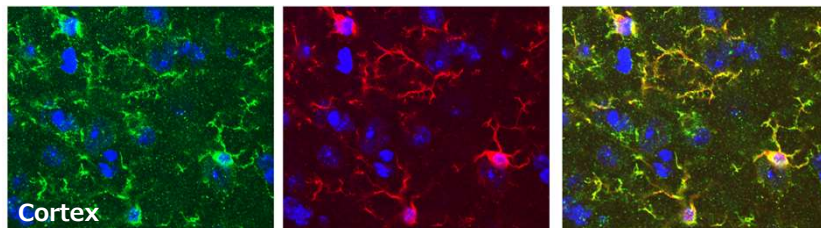
Our novel anti-P2RY12 antibody clearly recognized microglial cells, but not macrophages. We expect that guinea pig derived anti-P2RY12 antibody will be a useful tool that will facilitate future research breakthroughs toward a more accurate understanding of microglial function and characteristics.

Disclosure of Conflict of Interest

Matters requiring disclosures of COI with regard to the poster are as follows: Shiro Sugino, Daishi Hiratsuka, Satoshi Onodera, Masaaki Kojima are employees of FUJIFILM Wako Pure Chemical Corporation. Seiji Miyata and received consulting fees from FUJIFILM Wako Pure Chemical Corporation.

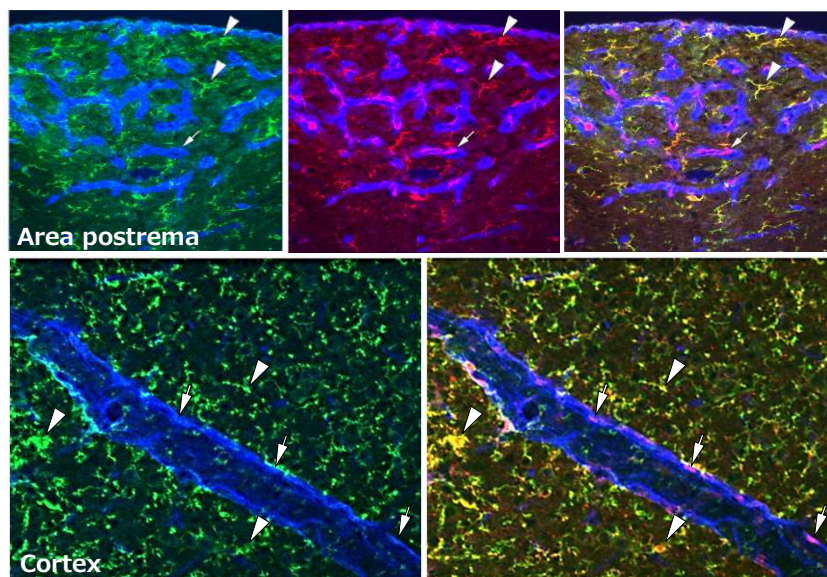
Application data (Immunohistochemistry)

1. Staining performance (P2RY12 vs Iba1 vs DAPI)



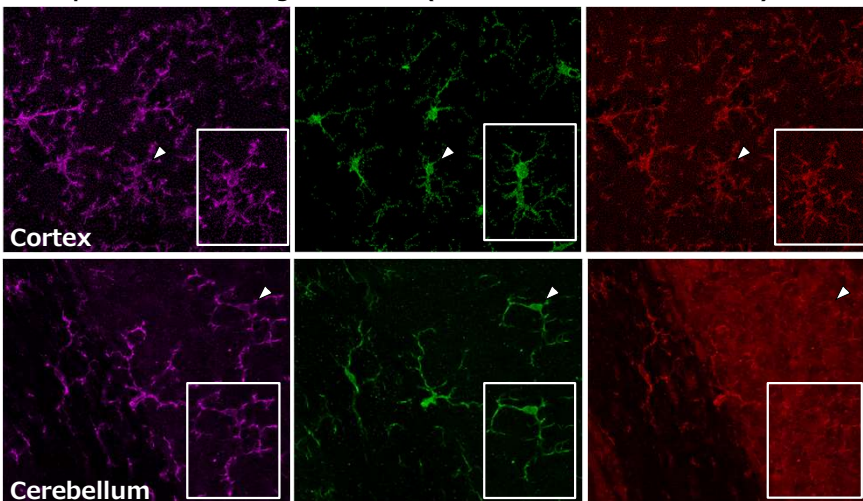
P2RY12 signals were detected in the cerebral cortex and hippocampus, all of which were localized in Iba1-positive microglia.

2. Distinction between microglia and macrophages (P2RY12 vs Iba1 vs Laminin)



P2RY12⁺ and Iba1⁺ microglia (arrowheads) were observed in the brain parenchyma. On the other hand, P2RY12⁻ and Iba1⁺ macrophage-like cells (arrows) were present in the perivascular area visualized in laminin-stained cryosections.

3. Comparison with microglial markers (P2RY12 vs Iba1 vs TMEM119)



The P2RY12 signals were also observed at the projection ends of microglia and detected fine projection structures were clearly detected compared to the Iba1 and TMEM119 signals.