F4/80 Antibody Mini Review
The marker and the antibody

Mini Review

F4/80

F4/80 is a well-characterized and extensively referenced membrane protein and is the best known mature mouse macrophage and microglial marker. The anti-F4/80 antibody clone CI:A3-1 is the original monoclonal produced against the F4/80 antigen in Siamon Gordon's laboratory at the University of Oxford (Gordon and Austyn, 1981).

Today, Bio-Rad is the only commercial manufacturer of the original clone of this important monoclonal macrophage antibody.

Thirty formats/sizes of F4/80 antibody products are currently available, from purified, and low endotoxin versions to a wide range of popular fluorescent labels. These fluorescent labels are suitable for flow cytometry and include choices such as FITC, RPE, DyLight® and Alexa Fluor® dyes, as well as the exclusive tandem conjugates RPE-Alexa Fluor® 647 and RPE-Alexa Fluor® 750.

Characteristics of the anti-F4/80 antibody clone CI:A3-1

In addition to recognizing kupffer cells, langerhans cells, peritoneal macrophages, and splenic red pulp macrophages, the **anti-F4/80 antibody** clone CI:A3-1 is distinguished from other F4/80 monoclonal antibodies in that it stains microglia (macrophages of the central nervous system) (Lawson *et al*. 1990). This may indicate that clone CI:A3-1 recognizes a different F4/80 epitope from other F4/80 antibody clones.

In addition to its extensive use in flow cytometry, clone CI:A3-1 is also suitable for functional studies and is available in a low endotoxin format for this purpose. The rat anti-mouse F4/80 antibody clone CI:A3-1 is also paraffin-reactive, allowing for immunohistochemical studies of paraffin-embedded sections of brain and other tissues.

Hundreds of scientific references support the use of the antibody clone CI:A3-1 in a wide range of applications and for a variety of experimental studies. Despite intensive investigation, however, the function of the F4/80 antibody target protein is not fully understood.

F4/80: gene and protein

F4/80 is known under several alternate names: Ly71, Gpf480, TM7LN3, DD7A5-7, EGF-TM7, and EMR1.

The F4/80 gene is located on mouse chromosome 17 and encodes a polypeptide of 931 amino acids that is processed into a mature protein of 904 amino acids. The predicted mass of the protein is 98.9 kDa, but the F4/80 protein runs at an apparent weight of 160 kDa in SDS-PAGE. The mass difference is probably accounted for by extensive glycosylation. Two papers describe the early cloning of the gene and the characterization of the protein in 1996 and 1997, McKnight *et al*. and Lin *et al*. respectively.

The N-terminus of the F4/80 polypeptide contains seven tandem EGF-like domains and shows a high degree of homology to proteins such as fibrillin-1 and fibulin-2. The C-terminal sequence demonstrates homology to members of the TM7 superfamily, such as the G protein-coupled receptors for peptide hormones, e.g. parathyroid hormone, calcitonin, and glucagon (McKnight *et al*. 1996).

The later discovery of other proteins with the same domain arrangement defined a new protein family named EGF-TM7, whose members include **F4/80, EMR1** (the human F4/80 homolog), and **human CD97** (McKnight *et al*. 1998).

The presence of an adhesion and a signaling domain in the F4/80 sequence suggests that it functions as a signaling molecule upon binding with other as yet unidentified cell-surface proteins. Ligands identified for other members of the EGF-TM7 protein family support this hypothesis, making it reasonable to conclude that F4/80 could have similar adhesion and signaling properties (McKnight *et al*. 1998).

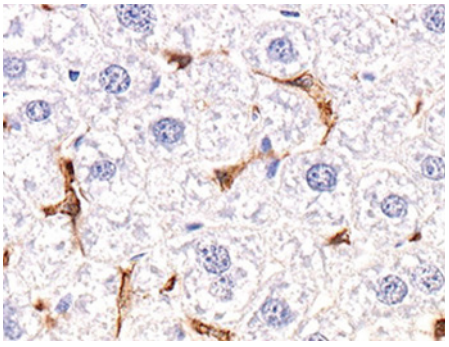

Fig 1. Liver tissue was fixed then stained with MCA497 after pre-treatment with citrate buffer at ph 6.0. Brown patches represent stained macrophages.

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F4/80: functions

F4/80 knockout mice appear vital, healthy, and fertile. The development and anti-microbe activity of macrophages in these animals also seems to be normal (Schaller *et al.* 2002).

However, Warschkau and Kiderlen (1999) have shown that administration of the anti-F4/80 antibody inhibits the production of cytokines (namely TNFa, IL-12, and IFN) from whole spleen cell cultures of SCID mice exposed to heat killed Listeria monocytogenes. Modulated cytokine response is believed to be dependent on the interaction of NK cells and macrophages in culture.

F4/80 is also expressed by eye-derived antigen-presenting cells (APCs) involved in anterior chamber-associated immune deviation (ACAID), a process that elicits tolerance for an antigen inoculated into the eye. ACAID is known to be antigen specific (Wang *et al.* 2001). Lin *et al.* (2005) went on to demonstrate the involvement of F4/80 in ACAID, in particular in the generation of efferent T regulatory (Treg) cells that are the effectors of tolerance.

Without knowing the cognate ligand of F4/80, Lin *et al.* (2005) could not describe a molecular mechanism for the involvement of F4/80 in ACAID, but they hypothesized that F4/80 is implicated in the interaction between tolerogenic APCs and NKT cells in the spleen that is essential for the development of the CD8+ Treg cells seen in ACAID.

In the same report, Lin *et al.* (2005) showed that F4/80 is also needed for CD8+ Treg cell induction in low-dose oral tolerance. Furthermore, F4/80 is expressed on APCs in the Peyer's patches (Makala *et al.* 2003) and on the majority of dendritic cells (DCs) in the central nervous system (Suther *et al.* 2003). These findings suggest that F4/80 could be involved in tolerance in systems distinct from ACAID and could be an essential part of the interaction between NK cells, macrophages, and **F4/80 expressing APCs**.

F4/80: expression

F4/80 is expressed at high levels on the surface of various macrophages: **kupffer cells, splenic red pulp macrophages, microglia, gut lamina propria**, and **langerhans cells** in the skin. Macrophages of the connective tissue, heart, kidney, reproductive, and neuroendocrine systems also express F4/80 (Gordon and Austyn, 1981).

Although F4/80 is broadly represented in macrophages, its expression varies depending upon the state of maturation, developmental processes, and type of macrophage. Langerhans cells are known to down regulate F4/80 expression after antigen uptake. This down regulation precedes migration to the local lymph nodes and is in accord with the hypothesized adhesion and signaling function of the protein (Gordon and Austyn, 1981).

Monocytes that circulate in the bloodstream also express F4/80 on the surface, but the level is lower than on tissue macrophages, indicating another correlation between the level of F4/80 and the adhesion properties of the corresponding mononuclear blood cell (Gordon and Austyn, 1981).

F4/80: microglia

Clone CI:A3-1 ability to stain microglia is due to a unique and advantageous feature of F4/80, as several other antigens, such as CD4, are down regulated once they pass through the blood-brain barrier (Perry and Gordon, 1991).

F4/80+ microglia are found throughout white and grey matter. Separate regions of the parenchyma contain F4/80+ cells varying in morphology; this allows us to distinguish microglia from other macrophage populations in the choroid plexus and leptomeninges (Gordon *et al.* 1992). Lawson *et al.* (1990) were able to produce a map of the distribution and morphology of microglia in the adult mouse CNS due to F4/80 labelling.

Microglia are associated with a number of disorders such as Alzheimer's Disease, Multiple Sclerosis and Prion Disease, which means that CI:A3-1 is a key F4/80 antibody clone to use in neurological research.

Pow *et al.* (1989), found that F4/80+ microglia in the posterior pituitary selectively endocytose terminals of neuroendocrine cells containing oxytocin/vasopressin, indicating that hormonal stimulation could be a functional response of F4/80+ microglia.

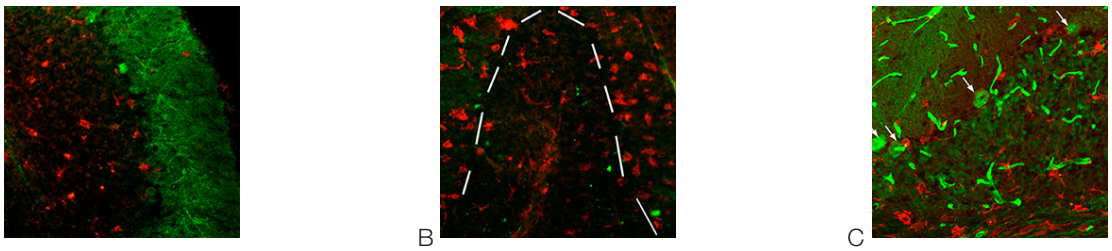

Fig 2. These images show sections from 50-d-old npc-1 mutant mice stained with anti-F4/80 antibody (red) with the aim of marking microglia. A) Shows an infiltration of microglia in the granule cell layer and white matter tract. B and C) Shows numerous microglia marked by anti-F4/80 present throughout the cerebellum, the dashed line indicating the edge of the granule cell layer (Dennis *et al.* 2005).

EMR1: the human homolog of F4/80

The human homolog of F4/80 was cloned and named EMR1 by Baud *et al*. (1995). EMR1 is located on chromosome 19 and its sequence shows 68% overall identity to F4/80. EMR1 also contains six EGF repeats and seven transmembrane segments supporting a purported function in adhesion and signaling that is in line with members of the EGF-TM7 family. Hamann *et al*. (2007) studied the expression and surface abundance of EMR1 using quantitative real-time PCR and flow cytometric analysis. They observed that EMR1 is surprisingly absent on mononuclear phagocytic cells, such as monocytes, macrophages, and myeloid dendritic cells. According to their report EMR1 expression is restricted to eosinophilic granulocytes, making it a highly specific eosinophil marker in humans.
While the absence of F4/80 on human monocytes and macrophages merits further investigation, the presence of F4/80 on eosinophils is not new to scientists. Murine eosinophils are known to stain with F4/80 and even Mac-1, but at lower intensity than is typical for macrophages and monocytes (McGarry *et al.* 1991). Bio-Rad offers anti-human EMR1 antibody for the study of the human equivalent of F4/80.

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