



# The Role of CD47 in Autoimmune Brain Inflammation

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## 1. ABSTRACT

CD47 (integrin associated protei is expressed in immune cells throughout the human body. Prior experiments show CD47 is downregulated for both mRNA and protein in active Multiple Sclerosis (MS) lesions. To assess its role in disease progression, we induced wild-type CD57/B6 mice with EAE. Data showed that mice treated with CD47 antibody at peak of disease exhibited a worsening of disease state with deviation in the cytokine profile.

Next, immunohistochemical studies were performed to decipher the expression of CD47 in three distinct subtypes of lesions: acute plaque, chronic active plaque, and chronic plaque. CD47 was found to be associated with compact myelin in normal tissue of diseases MS brains and decreased in demyelinated tissue of diseased MS brains. It was also expressed in reactive astrocytes and macrophages of both AP and CAP. We followed with in vivo assays of human myelin incubated with activated mouse macrophages in the presence of immunoglobulin as a control and CD47 antibody. In line with our hypothesis, results corroborated an increase in phagocytosis in cells exposed to the antibody. As CD47 interacts with the SIRPα receptor, a further study was performed using SIRPα antibody. This too showed increased phagocytosis. A confirmatory experiment was performed using splenocytes, in which similar results were seen.

The summation of these findings is that low levels of CD47 attenuate myelin phagocytosis, in turn, worsening demyelination. This study of CD47 exemplifies how a large scale profile can shed mechanistic insights into pathophysiology. While the focus here is on its impact in demyelination, this discovery opens the door to the possibility that CD47 is involved in creation of the packed myelin sheath around the axon. Furthermore, this discovery provides another direction for Multiple Sclerosis therapy.

## 2. BACKGROUND

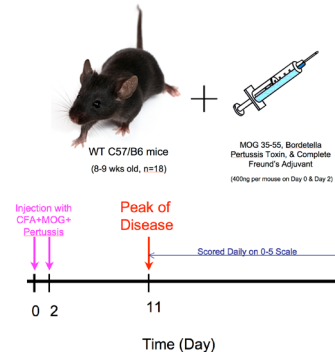
Proteomic and microarray analysis of brain lesions, cerebrospinal fluid and immune cells of MS patients have revealed several molecules to study in determining the mechanism behind autoimmune inflammatory disease; however, limitations to each type of analysis exist. A combined approach enabled us to determine molecules that overlapped between studies. Out of the molecules identified, CD47 was chosen as the focus of our study based on its role in other inflammatory states. CD47 signals via receptors SIRPα and thrombospondin to promote a multitude of functions.

## 3. OBJECTIVE

To determine the role of CD47 in the CNS and the PNS using the EAE model, human brain MS lesions, and by in vitro assay.

## 4. METHODS

### EAE and Treatment with mAb CD47,



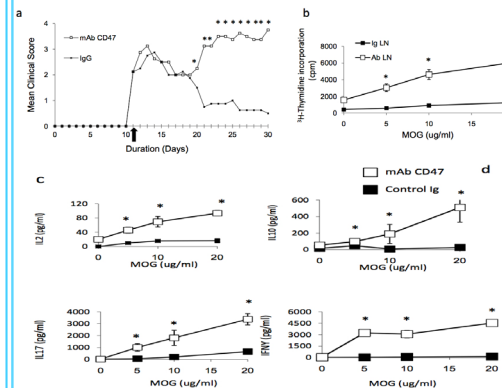
Wild type (WT) C57/B6 mice. EAE was induced in 8-9 weeks-old WT (n=18) immunized with Complete Freund's Adjuvant (CFA) and Myelin Oligoglycoprotein (MOG) peptide 35-55 and Bordetella pertussis toxin 400 ng per mouse on days 0 and 2. Mice were treated with daily intra-peritoneal injections of 100ug mAb CD47 or isotype control immunoglobulin (IgG) at the peak of disease. Mice were followed clinically every day up to day 35. Mice were scored according to: 0, normal; 1, tail paralysis; 2, hindlimb weakness; 3, complete hindlimb paralysis; 4, hindlimb paralysis with forelimb weakness and 5 moribund or death.

### Immune cell proliferation and cytokine analysis.

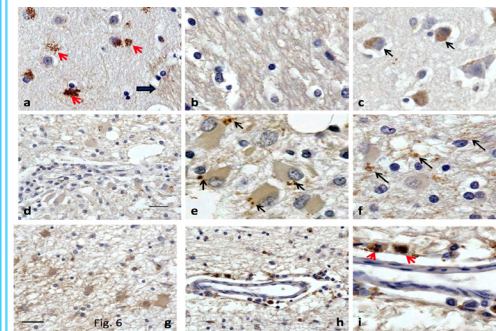
Splenocytes and lymph nodes cells from WT and CD47<sup>-/-</sup> mice were incubated in 96 well flat bottom plates in stimulation media and activated with increasing concentration of MOG35-55 peptide. For proliferation assays, cells were pulsed with 3H thymidine at 48 hrs and harvested 16 hrs later on a filter using a cell harvester and incorporated radioactivity was counted using a beta scintillation counter. For cytokine analysis immune cells were incubated for 24, 48, 72, 96 and 120 hrs and cytokine levels were measured at different time points utilizing anti-mouse ELISA kits (IL2, IL4, IL6, IL10, IL12, IFN $\gamma$ , OptEIA, BD Pharmigen, IL17, TNF, R&D Systems).

**In vitro phagocytosis assay.** Human myelin was isolated by mass spectrometry. Female C57BL/6 mice immunized subcutaneously with MOG35-55/CFA. Mice were given an intraperitoneal injection of Pertussis toxin at day 0 and day 2. Splenocytes were harvested on day 9. CFSE-labeled myelin fraction (1mg/ml) or EAE splenocytes were incubated with mouse macrophages in the presence of 10ug/ml Ig2a isotype, anti-mouse CD47 (m1AP301) or anti-SIRP $\alpha$  for 2 hr. Cells were analyzed to determine phagocytosis index (number of cells phagocytosed by 100 macrophages) by fluorescence microscopy. p<0.05 was determined to be statistically significant by Student's t-test. **Statistical Analysis.** Data represent means +/- s.e.m. A t-test was used for parametric data. Mann-Whitney U test was performed to detect between group-differences. A p value of <0.05 was rendered statistically significant.

## 5. RESULTS

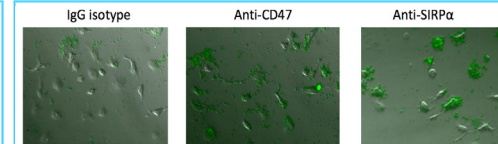


**Figure 1.** Treatment of EAE mice at peak of paralysis with CD47 Ab. a) Mean clinical score for Day 0-30 of EAE mice with treatment. b) Immune cell proliferation. c-f) Cytokine production of IL-2, IL-10, IL-17, and IFN- $\gamma$ .

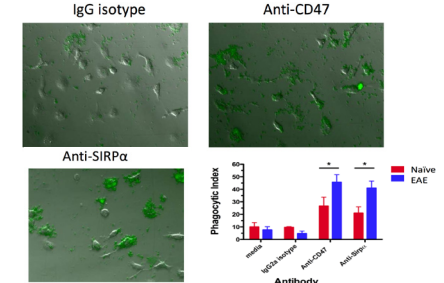


**Figure 2.** Immunohistochemistry of CD47 expression. a-c) In unaffected areas of MS brain, specifically in white matter tracts (a, arrow) and cortical neurons (c, arrow). d-f) In active plaques, CD47 expressed in myelin and reactive astrocytes, but was relatively absent in infiltrating immune cells. Ingested myelin (e, arrow) and myelin debris (f, arrow). g-h) In chronic active plaques, demyelinated regions showed less CD47 expression. Expression of CD47 remained high on macrophages and reactive astrocytes. i) Ingested myelin in chronic active plaques.

## 5. RESULTS



**Figure 3.** In vitro assays demonstrate blocking CD47 promotes phagocytosis of myelin. Human myelin was incubated with activated mouse macrophages in the presence of a) immunoglobulin, b) CD47 antibody, or c) SIRP $\alpha$  antibody.



**Figure 4.** In vitro assays demonstrate blocking CD47 promotes phagocytosis of splenocytes. Splenocytes from EAE mice were coated with either a) immunoglobulin, b) CD47 antibody, or c) SIRP $\alpha$  antibody and incubated with mature mouse macrophages. d) Phagocytic index

## 6. CONCLUSIONS

- § Treatment of EAE mice at peak of paralysis with CD47 monoclonal antibody worsens inflammation with enhancement of inflammatory cytokine production.
- § There is increased CD47 expression in the astrocytes and macrophages of both acutely active plaques and chronically active plaques.
- § Phagocytosis of myelin is dependent on the interaction between CD47 and SIRP $\alpha$ .
- § This phagocytic interaction is conserved in the CD47-SIRP $\alpha$  signal leading to phagocytosis of splenocytes.
- § Low levels of CD47 promote myelin phagocytosis resulting in worsening demyelination.

## 7. ACKNOWLEDGEMENTS

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