

# **Intra-subject analysis of LRP-1 expression in neurons and glia from both active and inactive MS lesions in comparison to unlesioned contra-lateral brain tissue**

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Background: Multiple Sclerosis (MS) is a chronic, inflammatory demyelinating disease of the central nervous system (CNS) of variable clinical course often beginning as a relapsing-remitting form and progressing into a secondary progressive disorder<sup>7</sup>. Remyelination of denuded axons in the CNS is usually rapid and efficient, and even MS lesions show some ability to remyelinate in remission stages of relapsing-remitting MS. However, as the pathological hallmark of chronic MS is demyelinated plaque, this repair process somehow eventually fails in MS patients<sup>8</sup>. The classical perception of MS is that it is a T-cell mediated autoimmune disorder initiated by the breakdown of the blood-brain barrier (BBB) leading to inflammatory cell infiltration and myelin destruction<sup>2</sup>. However, recent evidence suggests that immune cell infiltration and activation within lesion sites of the CNS may not be the primary cause of MS both as an initiator of chronic forms of the disease and as the trigger for new lesions in the relapsing-remitting form<sup>3,4,5</sup>. These recent studies indicate that widespread oligodendrocyte apoptosis and subsequent microglial activation can precede immune response indicating that, at least in some cases, immune cell infiltration and activation may actually be a response to demyelination and not the primary cause. Recent studies in our lab have demonstrated that low-density lipoprotein receptor-related protein (LRP-1) is a novel receptor mediating the phagocytosis of myelin debris in the CNS<sup>1</sup>. These studies also demonstrate that oligodendrocytes have the greatest expression of LRP-1 and the greatest capacity for the clearance of myelin debris. Therefore, widespread death of oligodendrocytes in a CNS lesion site would not only lead to myelin debris accumulation, but would greatly impair the ability of glial cells in that area to clear debris so remyelination could take place. Additionally, glial activation that takes place upon oligodendrocyte apoptosis and precedes immune infiltration can also impede the ability of oligodendrocyte progenitor cells (OPCs) to migrate into the lesion site, which has been shown to have the capacity to restore function in inactive lesion sites<sup>2,5</sup>.

Open Questions: Because studies in our lab demonstrate that LRP-1 is essential for the clearance of myelin debris, is the immune response seen in MS possibly an essential response by the body to facilitate the clearance of myelin debris and would restoration of LRP-1 expression in lesion sites restore the ability of glial cells to maintain homeostatic function without the need for immune infiltration?

Hypothesis: Active and inactive MS lesions without any remyelination will have significantly lower LRP-1 expression than contra-lateral unlesioned CNS tissue. Additionally, compensatory LRP-1 expression by astrocytes and microglia could facilitate adequate debris clearance and bypass the need for immune infiltration thereby altering the progression of MS.

Experimental Approach:

**Aim 1.** *Analyze the regional expression of LRP-1 between lesioned CNS without remyelination and contra-lateral unlesioned sites in brain slices of MS patients.*

Brain slices from patients who died from complications of MS would be taken for immunohistochemical analysis of lesioned versus unlesioned LRP-1 expression. Ideally, prior to autopsy some form of imaging data would be acquired to identify those lesions that would be identifiable as active and inactive lesions, which sites would be subsequently utilized in data collection and correlated back to radiologic identification. What we expect to find is a tremendous decrease in LRP-1 expression in active lesions and a less severe decrease in the inactive lesions in comparison to contra-lateral unlesioned tissue. However, because immune infiltration will have likely taken place in many of our patients due to the length of time these lesions had persisted it is highly likely that we may see even greater LRP-1 expression in lesion sites. To account for this possibility lesion sites will also be analyzed for cellular markers of oligodendrocytes, microglia, astrocytes, neurons, and macrophages to identify the relative cellular composition and relative LRP-1 expression between lesioned and unlesioned CNS tissue. In situations where lesions contain similar or greater amounts of LRP-1 than control tissue we would expect to see that the great proportion of this would be coming from infiltrating macrophages to compensate for a loss of oligodendrocytes. In lesioned tissues that demonstrate significantly lower LRP-1 levels we would expect to see low level of macrophage infiltration and decreased oligodendrocyte density compared to contra-lateral controls.

**Aim 2.** *Use increased expression of LRP-1 by astrocytes and microglia via transgenics or viral induction to decrease the severity of disease in EAE mice.*

The EAE mouse model utilizes CNS myelin components bound to Freud's adjuvant to induce autoimmunity to CNS myelin which leads to demyelination. While this model of MS is not well suited for our proposed alternative pathology of MS, it does have the benefit of being the most widely studied animal model of MS. Because demyelination does occur, increased expression of LRP-1 by oligodendrocytes and microglia could facilitate enhanced clearance of myelin debris, thereby allowing for OPC migration and remyelination, which would lead to decreased severity of disease. To accomplish this transgenic mice over-expressing LRP-1 in astrocytes and microglia could be used in the EAE model and monitored for disease progression and overall demyelination. Alternatively, mice given intrathecal injection of LRP-1 expressing lentivirus could also be a useful tool. However, because primary oligodendrocyte death is not a characteristic trait of EAE, it is likely that LRP-1 expression may be spared. This would not be surprising considering the EAE model is not chronically progressive nor does it relapse after remission and most mice will regain much of their function after some time and go into remission. The sparing of oligodendrocytes may be explanatory of this phenomenon and is worth investigation in its own right.

Because the EAE model is predicated upon immune infiltration initiating demyelination, it might be difficult to evaluate our hypothesis effectively in this system. As a result, using another transgenic animal that spontaneously demyelinate in the CNS may be a better tool. The DM20 transgenic mouse is a model of MS in which normal development to about 3 months occurs followed by spontaneous demyelination and eventual death around 8-10 months of age<sup>6</sup>. In this animal model enhancing the ability of glial cells to clear myelin debris could potentially prolong survival and slow disease progression in a manner much more comparable to what we hypothesize the potential therapeutic benefit to be in human MS patients. Because lymphocytic infiltration is seen at later stages of this disease, evaluating the ability of increased LRP-1 expression in the CNS to delay immune infiltration could provide invaluable insight into the pathologic progression of at least some forms of MS and could provide a key piece of evidence to pursue novel therapies of MS outside the traditional utilization of immunosuppressants and immune modifying therapies.

With new evidence suggesting that the prevailing conception of MS as a condition initiated by autoimmunity to CNS myelin may not be accurate, at least in some cases, the evaluation of alternative pathology must be pursued. As a result, evaluating non-immune initiation of demyelination and failure of glia to clear myelin debris may provide significant insight into the more subtle pathology of MS and possibly other demyelinating diseases.

**Fig. 1** CNS glia express LRP-1. (A) RNA was isolated from oligodendrocytes, astrocytes and microglia. LRP-1 mRNA expression was determined by qPCR (mean  $\pm$  s.d.) (B) Protein extracts from oligodendrocytes, astrocytes and microglia were analyzed by immunoblot with LRP-1  $\alpha$ -chain-specific antibody 11H4 and with antibody specific for ERK/MAP kinase as a control for load. The same samples also were analyzed by RAP-ligand blotting to detect LRP-1  $\alpha$ -chain and possibly other LRP family members. (C) MVs were analyzed by immunoblot using specific antibodies that detect MBP or LRP-1  $\alpha$ -chain and by RAP ligand-blotting. oligodendrocyte extracts were assessed in the same experiments. (D) PEA-10 and MEF-2 cells were incubated with FITC labeled-MVs for 30 minutes in the presence of GST or GST-RAP. After washing and protease treatment, cells were subjected to flow cytometry analysis.

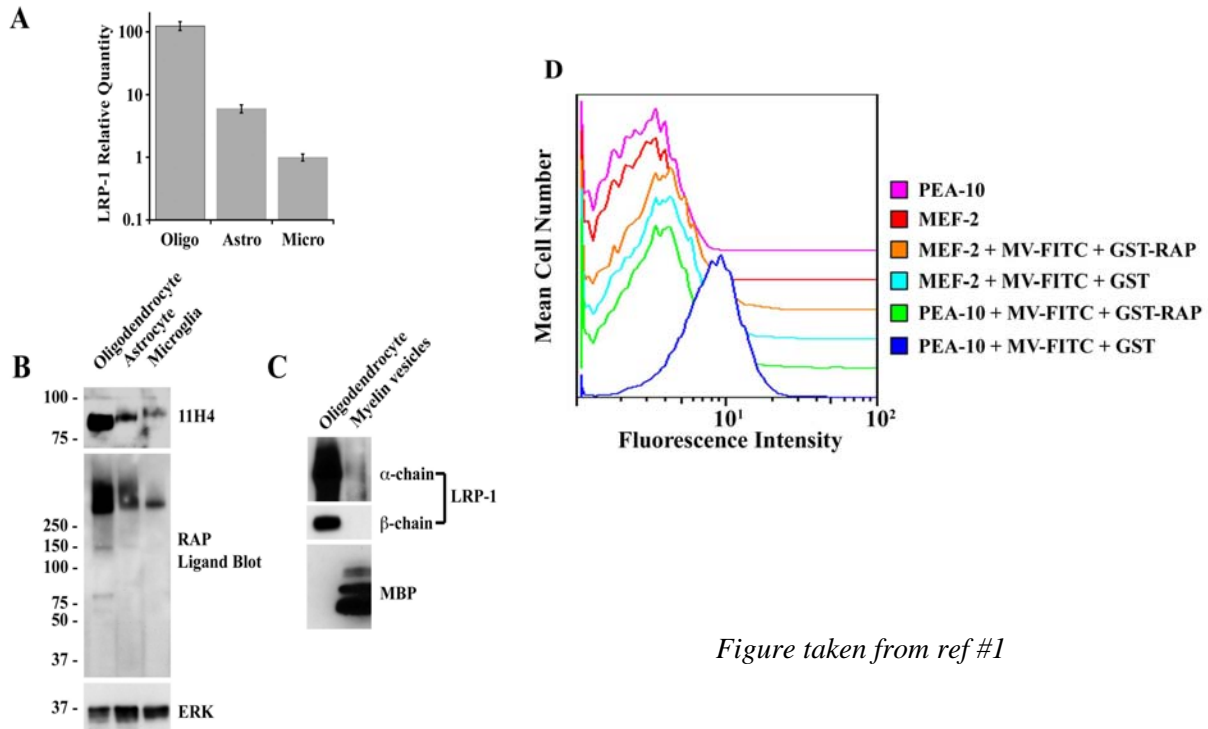


Figure taken from ref #1

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