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Discovery of remyelination therapeutics that overcome the effects of inflammatory macrophages on oligodendrocyte formation

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Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease that results in the disruption of neuronal transmission and ultimately neurodegeneration. Current treatments focus on suppressing the immune system to limit inflammation and loss of the myelin sheath. The next advance in the treatment of MS has focused on molecules that promote remyelination in order to restore function. One important question is how inflammatory factors in the lesion environment impact OPC differentiation and remyelination.

Macrophages are an innate immune cell known to infiltrate the CNS and accumulate in MS lesions. Macrophages release numerous factors that negatively impact OPC differentiation and remyelination efficiency. For example, these cells produce and release acetylcholine and lysophosphatidic acid (LPA), two factors we have shown inhibit OPC differentiation. Further, we have shown that these inhibitory effects are mediated by activation of both muscarinic M1 and LPA1 receptors located on OPCS. Based on these findings, we discovered and developed brain penetrant, selective, small molecule antagonists against M1 (PIPE-307) and LPA1 (PIPE-791). In the presence of their respective ligands, both small molecules antagonize their receptors, and permit OPC differentiation.

To better understand the impact of macrophages on OPC differentiation, as well as the contribution of these two receptors, we turned to a more complex, transwell culture system whereby OPCs are cultured in one compartment, macrophages in another, but media is freely exchanged. There are two key advantages this system has over an isolated OPC culture, 1) invading immune cells release a multitude of factors at various concentrations into the OPC environment and thus better represents the disease state in contrast to OPCs alone, 2) some of the secreted inhibitory/ factors are labile and the physical presence of macrophages provides a constant, renewable source unlike conditioned media which can be depleted.

Using this system, we observed a significant increase in the number of oligodendrocytes with either PIPE-791 or PIPE-307, demonstrating that even in the presence of inhibitory macrophages, either mechanism alone is sufficient to overcome the repression of differentiation. Both are promising therapeutics to promote remyelination in people with Multiple Sclerosis. PIPE-307 is currently advancing in phase 2 clinical trials as a remyelination therapeutic for MS while PIPE-791 is entering IND enabling toxicity studies.

Macrophages and other immune cells are found in and around MS lesions

Figure 1a

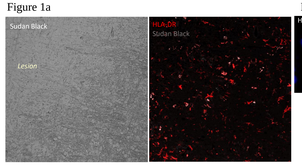

Figure 1b

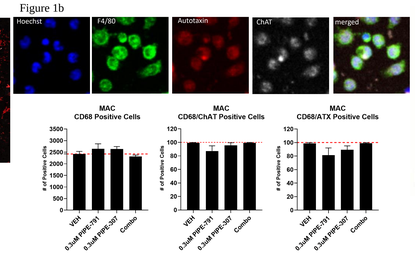

Figure 1a

Macrophages from peripheral tissues infiltrate into the CNS and contribute to the pathogenesis of MS.

Macrophages are found throughout active demyelinating MS lesions. These lesions contain an abundance of immune cells as evidenced by the presence of HLA-DR staining.

Figure 1b

Macrophages express two enzymes, ChAT and ATX, that are responsible for producing Acetylcholine and LPA. Male Sprague Dawley rat macrophages isolated from intraperitoneal lavage fluid were processed for immunocytochemistry against F4/80, ChAT and ATX. Top, images showing co-localization of Hoechst, F4/80, Autotaxin and Choline Acetyltransferase; bottom, quantification. Macrophages are not impacted by drug treatment.

Novel M1 and LPA1 antagonists induces Rat OPC differentiation

Figure 2a

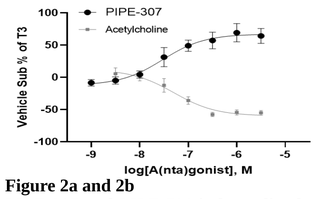

Figure 2b

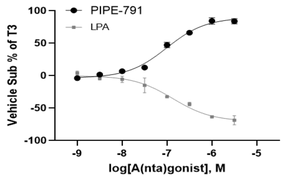

Figure 2a and 2b

PIPE-307 and PIPE-791 induce oligodendrocyte differentiation while Acetylcholine and LPA inhibit it in rat oligodendrocyte precursor cells (OPCs). OPCs isolated from rat postnatal day 8 cortex were treated with PIPE-307, Acetylcholine **(a)**, PIPE-791 or LPA **(b)** for 3 days following growth factor removal. Cells were PFA fixed and stained with MBP (mature oligodendrocytes) and Hoechst (DNA). Data are represented by vehicle subtracted percent of T3 control.

Macrophage addition suppresses baseline differentiation of OPC's to mature oligodendrocytes (MBP) in *Co-Culture* assay

Figure 3a

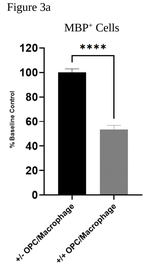

Figure 3b

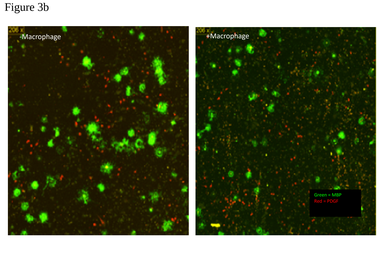

Figure 3a and 3b

Macrophage addition suppresses baseline differentiation of OPC's (PDGFRα)to mature oligodendrocytes (MBP) in Co-Culture assay. (a) OPCs isolated from rat postnatal day 8 cortex were co-cultured with rat macrophages for 72 hours. Cells were PFA fixed and stained with MBP (mature oligodendrocytes), PDGFRα (oligodendrocyte precursor cells) and Hoechst (DNA). Data are represented by vehicle subtracted percent of T3 control. T-test, **** $P \leq 0.0001$. **(b)** representative images.

Transwell culture system

Figure 4

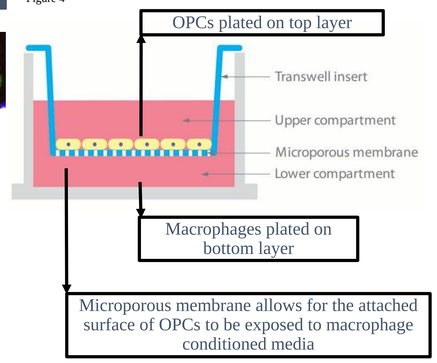

Figure 4

Transwell culture system. To circumvent the effect of cell-to-cell contact, we turned to a more complex, transwell system. Transwell culture systems are composed of an insert inside a 6-well plate. Insert is a 0.4 µm microporous membrane which allows for factors to flow between the top and bottom chamber. This permits us to minimize direct contact between the macrophage and OPCs while allowing for a constant, renewable source unlike conditioned media which can be depleted.

PIPE-307 and PIPE-791 overcome macrophage-mediated suppression both as single agents and in combination

Figure 5a

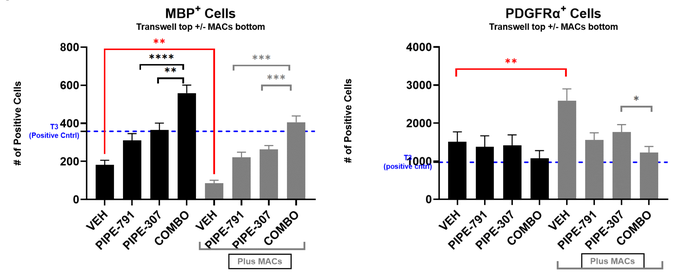

Figure 5b

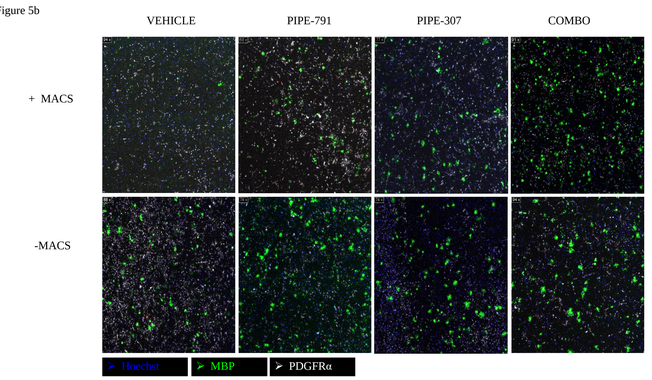

Figure 5a and 5B

PIPE-307 and PIPE-791 overcome macrophage-mediated suppression both as single agents and in combination (a) OPCs plated in transwell assay plates +/- macrophages on the bottom layer were treated with either PIPE-791 (0.3µM), PIPE-307 (0.3µM) or the combination. Cells were PFA fixed and stained with MBP (mature oligodendrocytes), PDGFRα (oligodendrocyte precursor cells) and Hoechst (DNA). Data are represented by the number positive cells. Vehicle treated OPCs +/- macrophages showed a statistically significant decrease in MBP positive cells and a subsequent decrease in PDGFRα positive cells. This effect was reversed with PIPE-791, PIPE-307 and the combination. **(b)** representative images.

PIPE-791 and PIPE-307 Improve Clinical Score and restores VEP latency in mouse EAE Model

Figure 6a

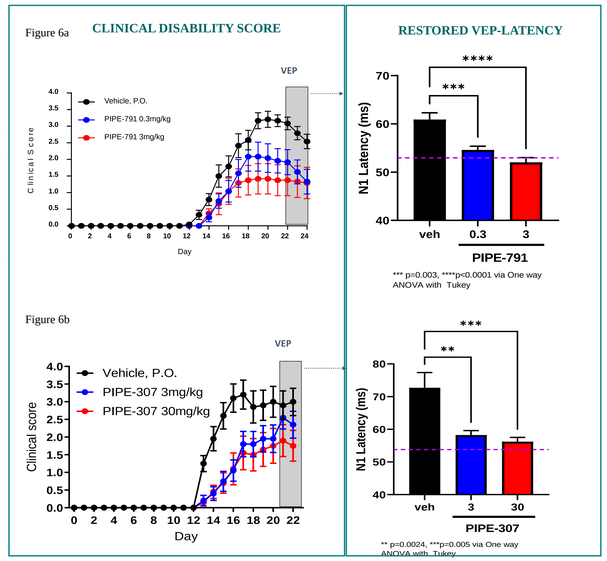

Figure 6b

Figure 6a and 6b

MOG-induced EAE leads to demyelination of the optic nerve and resulting increase in visual evoked potential latencies (VEP). These effects were reversed by treating animals orally with either **(a)** PIPE-791 (0.3 and 3 mg/kg) or **(b)** PIPE-307 (3 and 30 mg/kg).

Conclusion

- Multiple sclerosis (MS) is an inflammatory demyelinating disease that results in the disruption of neuronal transmission and ultimately neurodegeneration.
- Macrophages and other inflammatory cells infiltrate into the CNS and release numerous factors that negatively impact OPC differentiation and remyelination efficiency over the course of MS.
- Macrophages express choline acetyltransferase (ChAT) and autotaxin (ATX), the enzymes responsible for producing acetylcholine and lysophosphatidic acid (LPA). Both have been shown to inhibit oligodendrocyte differentiation and ultimately myelin production.
- Pipeline Therapeutics has discovered PIPE-307, a novel first-in-class M1R selective antagonist and PIPE-791, a novel brain penetrant LPA1 selective antagonist. Both have been shown to induce oligodendrocyte differentiation in healthy rat OPCs.
- Using a transwell assay system, we have shown that macrophages release diffusible factors that suppress OPC differentiation. This is reversed by both PIPE-307 and PIPE-791 as single agents and differentiation is augmented when both mechanisms are used in combination.
- PIPE-307 and PIPE-791 have been shown to produce robust effects in several *in vivo* models of MS including VEP and MOG EAE.
- Data suggests that infiltrating cells in MS lesions release several inhibitory factors (e.g. ACH and LPA) highlighting the potential value of targeting multiple mechanisms