THE GEORGE WASHINGTON UNIVERSITY
WASHINGTON, DC

HIV-1 NEF IMPAIRS MYELIN AND DISRUPTS GLIAL CELLS IN THE CENTRAL NERVOUS SYSTEM

00401

Jessica K Schenck[1], Robert H Miller[1], and Michael I Bukrinsky[1]
[1]The George Washington University, Washington, DC, USA

Extracellular vesicles containing the **HIV-1 protein Nef** impair **myelin** and disrupt **oligodendrocytes** in the Central Nervous System. These effects were attenuated by compounds *AMS-55* (Nef-Calnexin inhibitor) and *TO-901317* (LXR agonist), pointing towards ***ABCA1 dysregulation*** as the mechanism of exNef-mediated demyelination and a ***potential therapeutic target*** for HIV-Associated Neurocognitive Disorders.

BACKGROUND

HIV-Associated Neurocognitive Disorders (HAND) pathogenesis has been linked to white matter damage, but the mechanisms underlying HIV-associated demyelination remain unknown. We have shown that HIV-1 Nef is secreted from cells in extracellular vesicles (EVs) and impairs cholesterol efflux from macrophages via the cholesterol transporter ABCA1. Since oligodendrocytes require cholesterol during myelination, the current study examined the effects of exogenous Nef EVs on myelin structure, glial cell disruption, and ABCA1 expression in the central nervous system (CNS).

METHODS

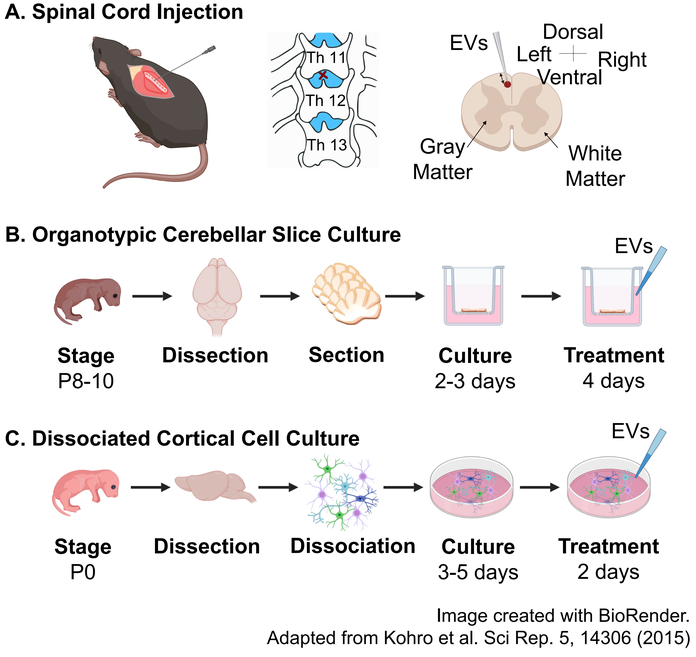


Fig 1. Methodology. Purified extracellular vesicles (EVs) containing recombinant Nef (exNef) or empty vector (exControl) were produced from transfected HEK293T cells. **A.** EVs were directly injected into the dorsal spinal cord white matter of adult mice; tissue was collected 72 h post injection. **B.** Cerebellar slices were obtained from P8-10 mice and cultured for 3-5 d, then treated with EVs once daily for 4 days. **C.** Primary cortical cultures were obtained from P0 Sprague-Dawley rats or C57BL6 mice, cultured up to 5 d, then treated with EVs once daily for 2 d.

RESULTS

In Vivo Myelin Analysis – Spinal Cord Injections

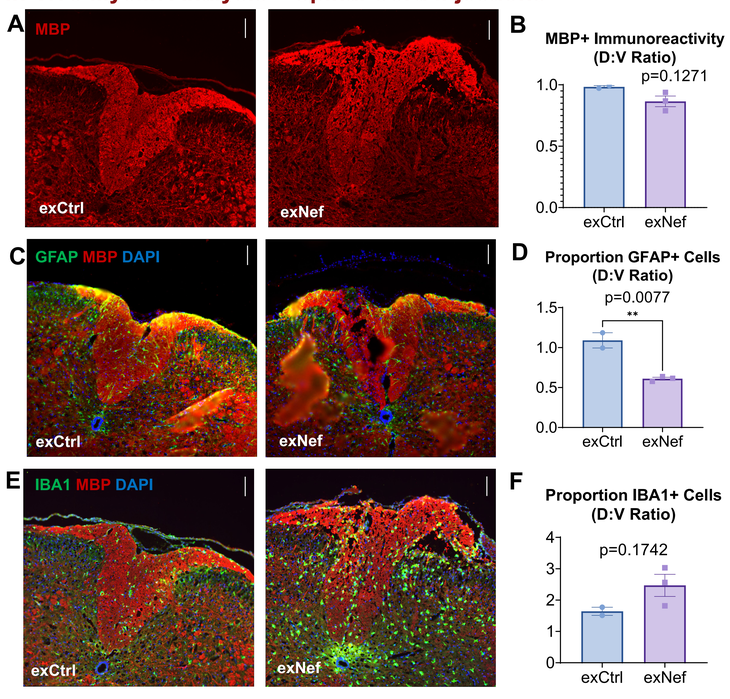


Fig 2. exNef disrupts myelin integrity *in vivo*.
Mouse dorsal spinal cords injected with exNef display:
A,B. decreased MBP+ myelin immuno-reactivity;
C,D. significantly decreased proportion of GFAP+ astrocytes;
E,F. increased proportion of IBA1+ microglia/myeloid cells.

For each sample, the injected dorsal column white matter was compared to the non-injected ventral column white matter. Scale bar set to 100 µm.

Ex Vivo Myelin Analysis – Cerebellar Slice Cultures

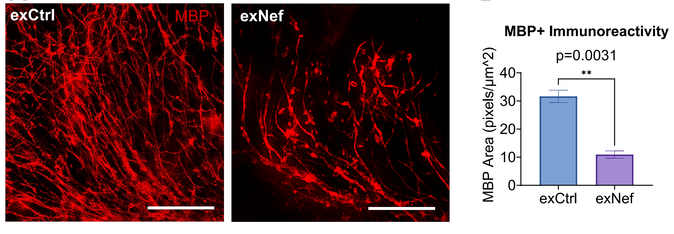


Fig 3. exNef disrupts myelin integrity ex vivo. A. Distinct alterations to myelin integrity in exNef-treated cerebellar slices compared to controls. **B.** Significant decrease in MBP+ immunoreactivity in slices treated with Nef EVs compared with controls. Scale bar set to 100 µm.

In Vitro Oligodendrocyte Analysis

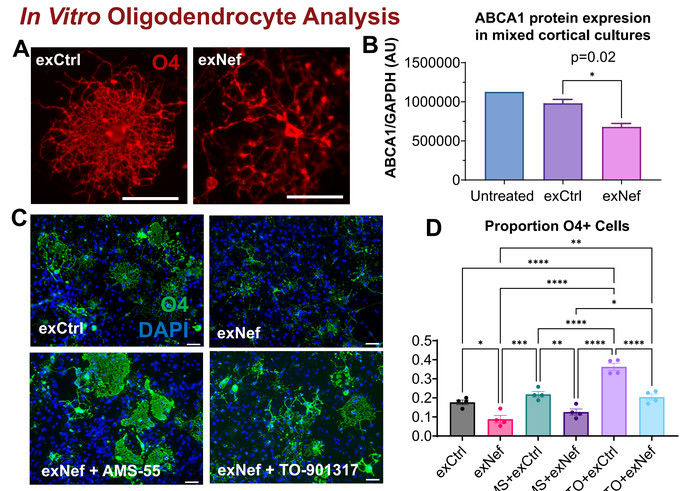


Fig 4. exNef disrupts oligo-dendrocytes and decreases ABCA1 protein expression.
A. Mixed rat cortical cultures treated with exNef contain O4+ pre-myelinating oligodendrocytes with altered processes compared to exCtrl. **B.** Mixed mouse cortical cultures treated with exNef showed significant reduction in ABCA1 protein expression compared to exCtrl. **C, D, E:** The proportion of O4+ cells and mean O4+ immunoreactivity was decreased in mixed mouse cultures treated with exNef; these effects were attenuated with drugs AMS-55 (Nef-Calnexin inhibitor) and TO-901317 (LXR agonist). Scale bar set to 50 µm.

CONCLUSIONS

We found exNef: 1. Altered MBP+, GFAP+, and IBA1+ immunoreactivity at injection site *in vivo*, 2. Decreased MBP+ immunoreactivity *ex vivo*, 3. Disrupted O4+ cells and decreased ABCA1 protein expression in *vitro*. Further, AMS-55 and TO-901317 attenuate exNef damage to O4+ cells *in vitro*. **Therefore, Nef extracellular vesicles disrupt myelin integrity and impair mature oligodendrocytes in the CNS, potentially mediated by ABCA1 dysregulation.**

ADDITIONAL KEY INFORMATION

Research reported in this poster was supported by the American Heart Association under Predoctoral Fellowship award number 20PRE35080036. We thank the GWU Institute for Biomedical Sciences and Department of Microbiology, Immunology, & Tropical Medicine for their continued support.