Mechanisms of White Matter Loss due to HIV Infection and Antiretroviral Therapy

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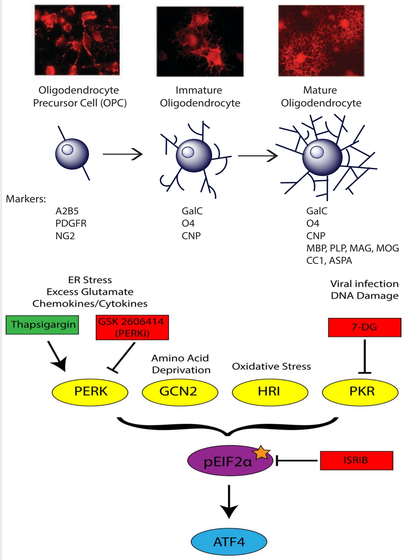
Background

White matter pathologies including corpus callosum thinning and disruption of white matter microstructures persist in HIV-positive patients on antiretroviral (ARV) therapy (ART) (Tate et al., 2011). Thinning of the corpus callosum increases with time on ART (Jernigan *et al.*, 2011). Interestingly, a transcriptome analysis comparing untreated and ART-treated patients with HAND showed downregulation of genes critical for oligodendrocyte differentiation and myelin production in patients with HAND regardless of ART (Borjabad *et al., 2011*). These findings suggest that ART as well as HIV may contribute to the observed white matter deficits in HAND.

We hypothesize that HIV-infected macrophages and/or antiretroviral compounds alter oligodendrocyte differentiation, function, and/or survival and sought to identify the underlying mechanisms.



Scheme 1. Oligodendrocyte lineage cells follow a specific maturation organization of transiently expressed transcription factors and cellular proteins. We can monitor these markers to evaluate maturation from oligodendrocyte precursor cells (OPCs) to immature and mature oligodendrocytes. We use galactocerebroside (GalC) to mark immature oligodendrocytes and myelin basic protein (MBP) for mature oligodendrocytes.

Scheme 2. Schematic of the integrated stress response (ISR), which is activated in response to extrinsic and intrinsic stressors such as viral infections and accumulation of unfolded proteins in the endoplasmic reticulum (ER), respectively. ISR activation involves **phosphorylation of eukaryotic initiation factor 2 alpha (eIF2α)** which attenuates global protein translation while promoting translation of certain stress response proteins including **activating transcription factor 4 (ATF4)**. ISR is activated in astrocytes and neurons of HIV+ and HAND individuals (*Akay et al. 2012*). Thapsigargin is an ISR inducer used as a positive control. 7-DG and ISRIB are inhibitors of PKR and pEIF2α, respectively.

Results

Figure 1. HIVMDMs treatment inhibits OL differentiation, which is attenuated by ISR inhibition

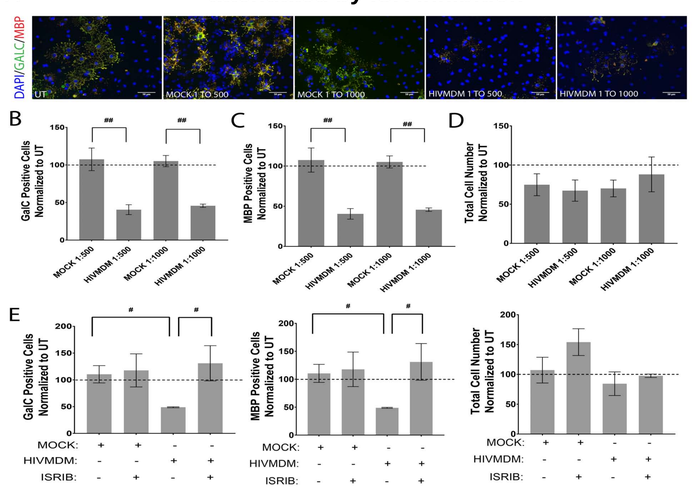


Figure 1. Primary rat OPC cultures were treated for 72 hours with supernatants of HIV-infected or mock-infected monocyte-derived macrophages (HIVMDMs and Mock, respectively) and stained for the OL markers GalC (green) and MBP (red) by immunofluorescence. A) Representative images of oligodendrocytes stained for GalC (green), MBP (red), and DAPI (blue for nuclei) in untreated, Mock-treated, and HIVMDM-treated cultures from one donor. Labeled cells were hand-counted and expressed as percentages relative to untreated (UT). B) Immature oligodendrocytes (GalC+ cells). C) Mature oligodendrocytes (MBP+ cells). D) Total cells (DAPI+ cells) N = 3, #P<0.05, ##<0.01, ###P<0.001, ####<0.0001 (One-way analysis of variance, Dunnett's *post hoc*). E) The ISR inhibitor trans ISRIB (ISRIB) attenuates the loss of GalC- or MBP-positive mature oligodendrocytes. N = 3, #P<0.05, ##<0.01, ###P<0.001, ####<0.0001 (One-way analysis of variance, Dunnett's post hoc).

Results

Figure 2. The integrase inhibitor elvitegravir (EVG) inhibits OPC differentiation, which is mitigated by ISR inhibition

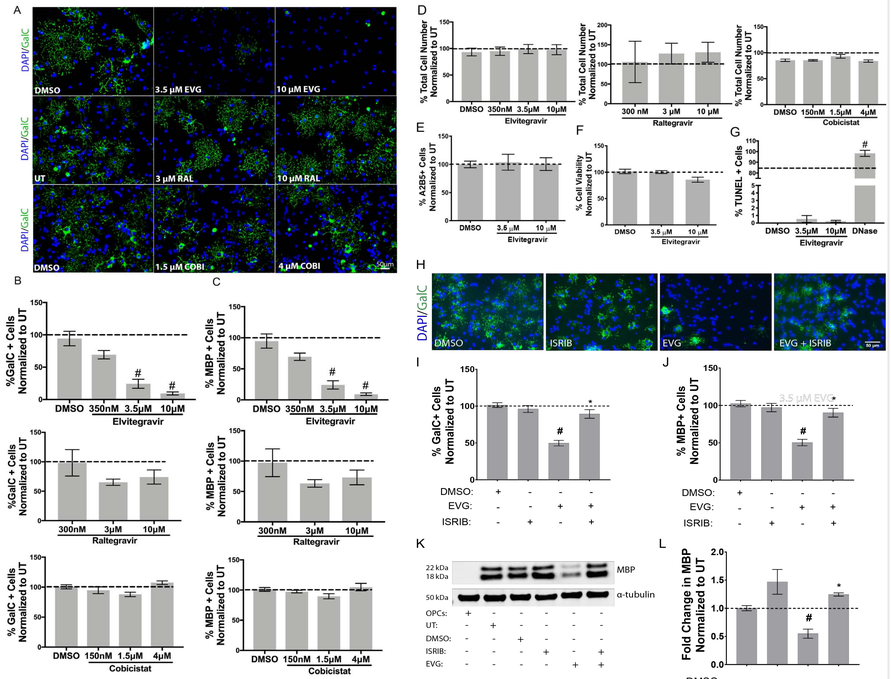


Figure 2. (A-G) Primary rat OPCs plated on coverslips or petri dishes were put into differentiation medium and treated with Vehicle (DMSO), EVG (350 nM, 3.5,or 10 μM), cobicistat (COBI 150 nM, 1.5, or 4 μM) or raltegravir (RAL, 300 nM, 3.0, or 10 μM). After 72 hours cells were fixed and stained for GalC (green), MBP (red), and DAPI (blue) . Cells were counted using Fiji software, represented as percentage normalized to untreated. A) Representative immunofluorescent images. B) Immature oligodendrocytes (GalC+ cells). C) Mature oligodendrocytes (MBP+ cells). D) Total cells (DAPI+ cells). E) OPCs (A2B5+ cells). F) Cell viability (identified as cells negative for propidium iodide) G) Apoptotic cells (identified as TUNEL+ cells). (H-L) Primary rat OPCs plated on coverslips or petri dishes were put into differentiation medium and pretreated with trans-ISRIB (5 μM) for 1 hour prior to EVG (3.5 μM) treatment. Coverslips were processed as described for (A). H) Representative images. I) Immature oligodendrocytes (GalC+ cells). J) Mature oligodendrocytes (MBP+ cells). K) Representative immunoblot for MBP and the loading control,α-tubulin. L) Quantification of MBP is fold change from untreated (UT). N = 3, #p<0.05, compared with DMSO control. *P<0.05, compared with EVG alone. (One-way analysis of variance, Tukey's *post hoc*).

Figure 3. EVG inhibits remyelination *in vivo*

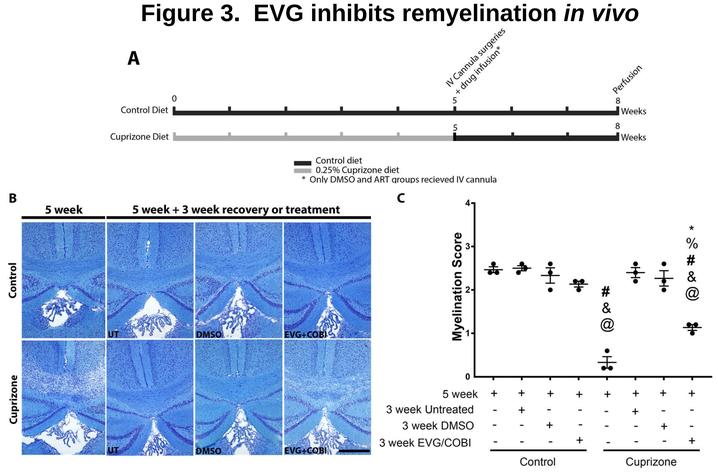


Figure 3. To examine the effect of ARV drugs on remyelination, we treated mice with cuprizone, a demyelinating compound, for five weeks and allowed them to recover for three weeks or treated them with daily intrajugular injection of EVG plus COBI during the three-week recovery phase. Brains were harvested, and the corpus collosum was sectioned and stained for myelin by luxol fast blue (LFB), mature oligodendrocytes by ASPA and GFAP, and IBA1 as a marker for microglia to assess neuroinflammation. A) Scheme of the cuprizone demyelination model to assess the effect of EVG on remyelination *in vivo*. B) Representative images of mouse cortical sections stained with LFB for myelin. C) Myelination score based on blind-scoring of corpus callosum sections stained with LFB. A higher score reflects more myelination, whereas a lower score reflects the absence of myelination. Individual data points (circles) with standard error of means for three animals per group. Repeated-measures analysis of variance with Sidak *post hoc* (all compared with 5-w cuprizone + 3-w EVG/COBI): @ 5-w control p < 0.0001; # 5-w control + 3-w DMSO p < 0.0001; % 5-w cuprizone + 3-w untreated p < 0.0001; * 5-w cuprizone + 3-w DMSO p < 0.0001.

Results

Figure 4. EVG inhibits OPC differentiation and induces neuroinflammation *in vivo*

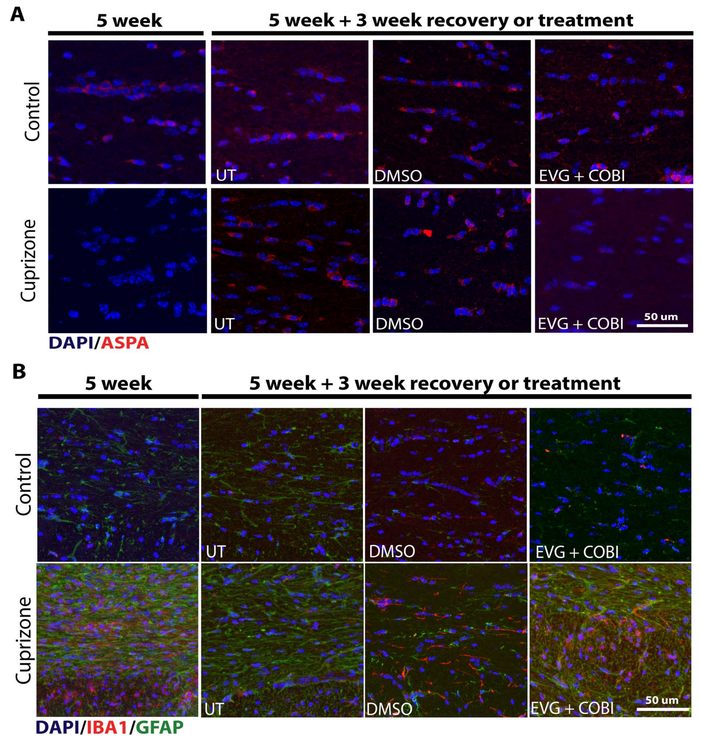


Figure 4. A) Representative images of mouse cortical sections stained with ASPA and DAPI, (blue, nuclei). B) Representative images of mouse cortical sections stained with GFAP, IBA1, and DAPI. C) Percentage of ASPA+ cells normalized to 5-w control. Individual data points (circles) with standard error of means for 3 animals per group. Repeated-measures analysis of variance with Sidak *post hoc* (all compared with 5-w cuprizone + 3-w EVG/COBI): & 5-w control + 3-w untreated p < 0.0001; # 5-w control + 3-w DMSO p < 0.0001; % 5-w cuprizone + 3-w untreated p < 0.0001; * 5-w cuprizone + 3-w DMSO p < 0.0001. D) GFAP fluorescent intensity. Individual data points (circles) and mean values (horizontal lines) are plotted for 3-5 animals per group. Repeated-measures analysis of variance with Sidak *post hoc* (all compared with 5-w cuprizone + 3-w EVG/COBI): @ 5-w control p = 0.004; & 5-w control + 3-w untreated p < 0.0001; # 5-w control + 3-w DMSO p = 0.0003; % 5-w cuprizone + 3-w untreated p = 0.0036; * 5-w cuprizone + 3-w DMSO p = 0.0001. E) IBA1 fluorescent intensity. Individual data points (circles) with standard error of means are plotted for 3 animals per group. Repeated-measures analysis of variance with Sidak *post hoc* (all compared with 5-w cuprizone + 3-w EVG/COBI): @ 5-w control p = 0.0003; & 5-w control + 3-w untreated p = 0.0002; # 5-w control + 3-w DMSO p = 0.0001; % 5-w cuprizone + 3-w untreated p = 0.0037; * 5-w cuprizone + 3-w DMSO p = 0.0057.

Conclusions & Future Directions

- HIVMDMs and elvitegravir treatment inhibit oligodendrocyte differentiation *in vitro,* whereas raltegravir and cobicistat do not.
- Trans-ISRIB attenuates HIVMDMs- and EVG-induced inhibition of oligodendrocyte maturation.
- EVG inhibits remyelination following cuprizone-induced demyelination *in vivo.*

Future studies will elucidate:

- The role of the ISR in recovery of oligodendrocyte maturation after HIVMDM treatment.
- Functional outcomes of oligodendrocyte dysfunction due to ART and HIVMDMs.
- Whether inhibition of the ISR can rescue EVG-induced myelination defect *in vivo*.

Evaluation of this work has been provided by Jordan-Sciutto and Grinspan laboratories.
This project was supported by R01 MH098742 to KJS and JBG and T32GM008076 to LMR.