Poster # 481

UC San Diego
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MBL-HIV gp120 Immunoreactivity is Associated with Markers of Neuronal Injury

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Background

Mannose-binding lectin (MBL), an innate immune response protein binds directly to the mannose residues on HIV-1 envelope glycoprotein 120 (gp120) via its carbohydrate recognition domain (CRD) and leads to complement activation and virus opsonization. We have shown that intracellular MBL mediates subcellular trafficking of HIV-1 gp120 in neurons (Teodorof et al, NBD, 2014). In this work, we hypothesized that MBL-gp120 interactions in HIV-1 infected brain will be associated with neuronal damage.

Methods

HIV-1 infected frontal cortex brain tissues with or without HIV encephalitis (HIVE) (obtained from California NeuroAIDS Tissue Consortium) and hemi-brain sections from wild type (WT) and gp120 transgenic (gp120tg) mice were used to analyze MBL expression and immunoreactivity with somatodendritic marker MAP2, neurodegeneration marker phospho-Tau (pTau), synaptic marker synaptophysin (SYF) and complement activation product C3d. Furthermore, human primary neuronal cultures (HPNs) were treated with 5nM IIIB-gp120 to determine its effects on neuronal damage. Immunohistochemistry and confocal microscopy were used to analyze expression and immunoreactivity of MBL and gp120 with neuronal markers.

FIG.1. LOSS OF MAP2 NETWORK IN HIV-1 IIIB GP120 TREATED HPNS

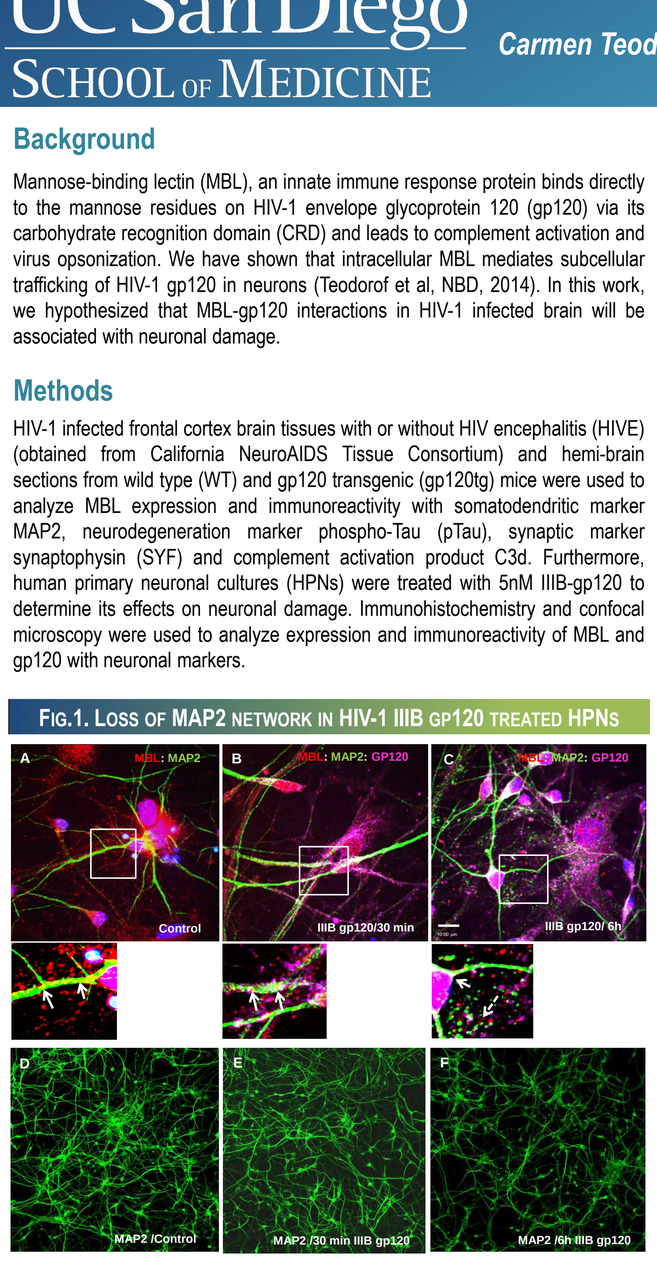


Fig.1 Degradation of microtubule network in HIV-1IIIBgp120 HPNs. Human primary neurons were untreated (A) or treated with IIIB gp120 (5 nM) for 30 min (B) and 6 h (C). Neurons were fixed and stained for MBL (red), MAP2 (green), gp120 (magenta) and DAPI for nuclei (blue). Localization of MBL:MAP2 (open arrows; yellow indicates co-localization) and MBL:MAP2:gp120 complexes (open arrows; white indicates co-localization) in neuronal soma and neurites. Dashed open arrows indicate fragmentation of the microtubules. Reduction of MAP2 somatodendritic network in HPNs treated with HIV-1 gp120 IIIB for 0, 30 min and 6 h (D, E, F, 20X). Scale bar, 10 µm.

Results

FIG. 2. LOSS OF SOMATODENDRITIC MAP2 IN HIVE BRAIN

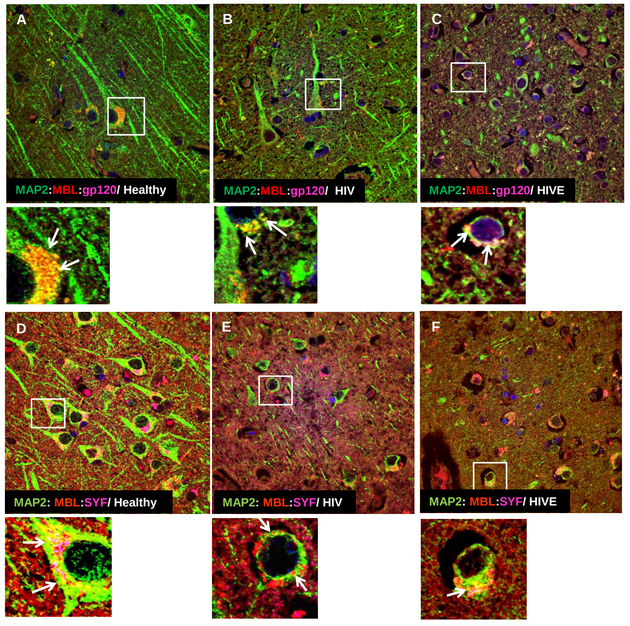


Fig.2 Somatodendritic damage and loss of MBL:MAP2:gp120/SYF immunoassociation in Human Postmortem Tissue from Normal (non-HIV) (A, D), HIV (B, E) and HIVE (C, F) subjects. Sections were stained for MBL (red) , MAP2 (green) and Synaptophysin/gp120 (magenta). MAP2:MBL:gp120 and MAP2:MBL:SYF immunecomplex association is indicated by the white arrows.

FIG. 3. LOSS OF SOMATODENDRITIC MAP2 AND SYF IN GP120 TG MICE

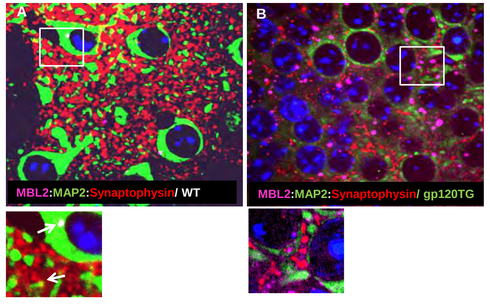


Fig.3 Somatodendritic damage and loss of MBL:MAP2:SYF/gp120 immuneassociation in gp120 TG mice brain. Brain sections of WT (A) and gp120 Tg (B) mice were stained for MBL2 (magenta) , MAP2 (green) and Synaptophysin (red). MAP2:MBL: SYF immunecomplex localization is indicated by the white arrows.

Results cont.

FIG. 4. LOSS OF SYNAPTIC PUNCTA IN HIV-1 IIIB GP120 TREATED HPNS

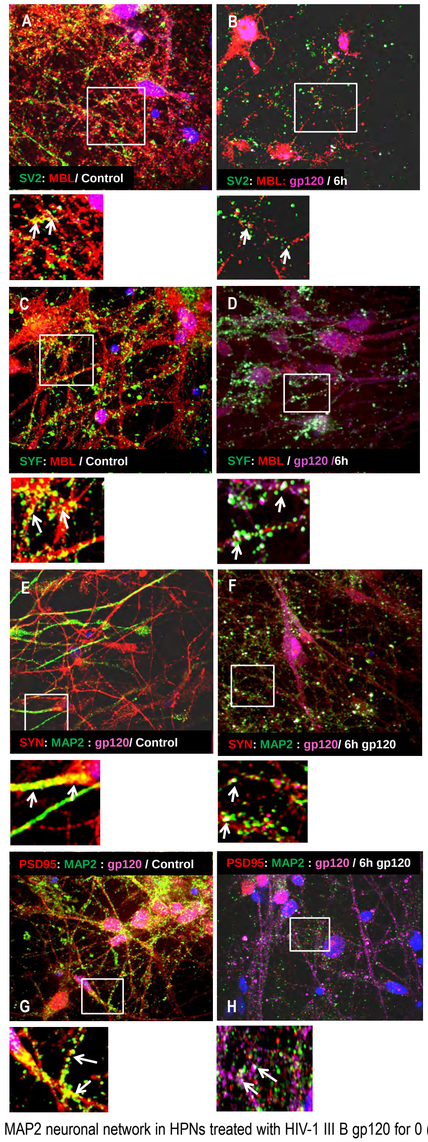


Fig.4 Loss of MAP2 neuronal network in HPNs treated with HIV-1 III B gp120 for 0 (A,C,E,G) and 6 h (B, D, F, H) is associated with reduction of synaptic markers (SV2, SYF, SYN, PSD95).. Neurons were stained for MBL/Synapsin /PSD95 (red), SV2, Synaptophysin (green) and gp120 (magenta). Immunecomplex association and localization are indicated by the white arrows.

Results cont.

FIG. 5. INCREASED EXPRESSION OF NEURONAL INJURY MARKER IN HIVE

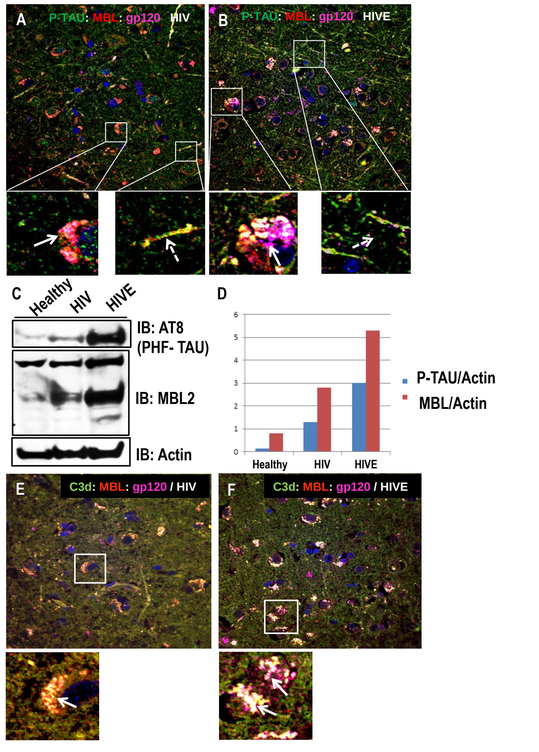


Fig.5. Increased Deposition of P-TAU and C3d in HIVE is Associated with Increased MBL Expression. Sections from HIV and HIVE were stained for P-Tau (green) (A,B) and C3D (green)(E,F) in combination with MBL (red) and gp120 (magenta). NFT (neurofibrillary tangles) formation is indicated by white dashed open arrows (A,B). P-TAU:MBL:gp120 and C3D:MBL:gp120 immunecomplex deposition are indicated by white open arrows. Expression of P-TAU (PHF-TAU) and MBL in human postmortem tissue (C) and quantification (D).

Conclusions

- Presence of MBL-gp120 immune complexes was associated with a degradation of MAP2 network and loss of SYN, SV2, Synapsin and PSD95 puncta in HIVE brain and gp120 TG mice hemi-brains. An *in vitro* model of human primary neurons confirmed the loss of MAP2 network in the presence of HIV1-gp120.
- Neuronal Injury in HIVE was indicated by increased expression of P-Tau and immune deposition of C3d, suggesting a unique role of MBL-gp120 interactions and complement activation in neuronal damage.

Acknowledgement

Authors acknowledge the support by National Institute of Health (NIH) R01 MH085608 and R03 MH103995 to KKS; MH087332, DA026306 to MK; and MH62512, DA26306 to CLA and BS.

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