

HMGB1 Immune Complexes Regulate Immune Responses to Alcohol

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Neuroimmune activation is a key feature of the pathologies of alcoholism and drug addiction (Cui, Shurtleff et al. 2014, Montesinos, Alfonso-Loeches et al. 2016). HMGB1 is an ethanol-induced immune mediator that can act as a chaperone to enhance signaling through various Toll-like receptors (TLRs) or cytokine receptors (Crews, Lawrimore et al. 2017). We investigated the ability of ethanol to induce the secretion of HMGB1/miRNA-let7b and other HMGB1/cytokine heterocomplexes leading to enhanced innate immune gene induction. Since HMGB1 has been shown to utilize an unconventional vesicular secretion method in other settings, we assessed the efficacy of an inhibitor of this pathway (Wortmannin, a PI3 kinase inhibitor) to prevent HMGB1 secretion from microglia.

Methods: *Postmortem Human Alcoholic Hippocampus* and *in vivo* mouse models: HMGB1 and TLR7 were measured in brain by ELISA and western blot. Mice underwent acute (6g/kg) or subchronic (5g/kg, 10 days) binge ethanol. Co-immunoprecipitation was used to detect HMGB1 heterocomplexes with certain cytokines. *In Vitro:* Induction of HMGB1, HMGB1 heterocomplexes, and the release of endogenous TLR7 agonist miRNA let-7b in microvesicles (MVs) by ethanol were assessed in Hippocampal-Entorhinal Cortex (HEC) Culture and BV2 microglia. The PI3K inhibitor wortmannin was tested for its ability to prevent HMGB1 release from microglia. RNA immunopurification (RIP) assay assessed let-7b binding to HMGB1 in MVs

Results: Ethanol induced TLR7 expression *in vivo* and *in vitro*. Ethanol pre-treatment enhanced TLR7 activation both *in vitro* (1.6-fold) and *in vivo* (2.7-fold). Ethanol caused secretion of the endogenous TLR7 agonist, miRNA let-7b, in microglia-derived microvesicles (MVs) and increased the binding of HMGB1 to miRNA let-7b in MVs. HMGB1 was required for TLR7-mediated neurodegeneration. Post-mortem human alcoholic hippocampal tissue showed increased levels of HMGB1/cytokine heterocomplexes. Ethanol induced a transient cytoplasmic vesicular association of HMGB1 with this cytokine *in vivo*. Ethanol increased the secretion of heterocomplexes from HEC slice culture. Recombinant HMGB1 heterocomplexes formed *in vitro* synergistically enhanced neuroimmune responses. The PI3K inhibitor Wortmannin prevented HMGB1 secretion in BV2 microglia in response to ethanol.

Discussion: These studies find novel neuroimmune mechanisms in the pathology of alcoholism. Immunogenic HMGB1 heterocomplexes represent novel targets for immune modulatory therapy in alcohol use disorders, and should be investigated in other psychiatric diseases that involve a neuroimmune component. Further, inhibition of the unconventional vesicular secretion pathway by wortmannin prevents HMGB1 release and may represent a novel immune therapy.

References: Crews, F. T., C. J. Lawrimore, T. J. Walter and L. G. Coleman (2017). *Neuropharmacology*. Cui, C., D. Shurtleff and R. A. Harris (2014). *Int Rev Neurobiol* **118**: 1-12. Montesinos, J., S. Alfonso-Loeches and C. Guerri (2016). *Alcohol Clin Exp Res* **40**(11): 2260-2270.

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