# Heme Induces Microglial CXCL2 Release - a Mechanism of Neutrophil-Mediated Injury after Intracerebral Hemorrhage

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## Background

Intracerebral hemorrhage (ICH) is a multifactorial and devastating injury to the CNS. Patients with ICH have a 30 day mortality rate of up to 40%, and persistent executive dysfunction is extremely common among survivors.

Recruitment of peripheral immune cells to the site of hemorrhage may play a role in the pathology of ICH. Neutrophils are recruited into tissue via CXCL chemokine expression, and while they play a critical role in debris removal, they may also be a source of inflammatory-mediated injury leading to long-term neurological deficits.

Heme, a metabolite of hemoglobin (Hgb), has recently been characterized as an endogenous ligand for Toll-like Receptor 4 (TLR4).

Activation of TLR4 by heme in the CNS initiates an immune response, recruits neutrophils to the site of injury, and leads to cognitive dysfunction due to inflammatory-mediated neuronal death - yet the molecular signals by which neutrophils are recruited to the site of hemorrhage have not been fully characterized.

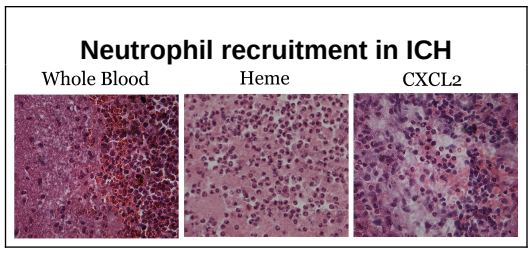


**Fig. 1.** Under stereotactic guidance, 25µL whole blood, heme (100µM) or CXCL2 (10µL, 0.1 µg/mL) was aseptically injected into the ventral striatum of rats at 2 µL/min. At 24 hours, the multilobed nuclei of neutrophils are readily visible on H&E. Note that all conditions led to prominent neutrophil invasion of the CNS.

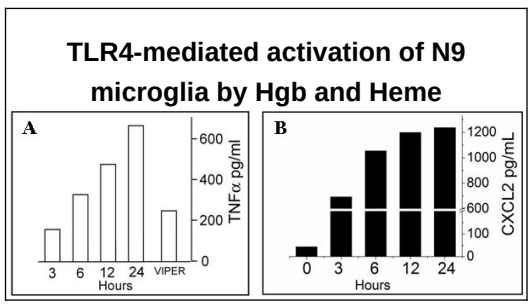


**Fig. 2.** ELISA assays for TNFa and CXCL2 detected in the media of N9 microglia (a transformed cell line) exposed to 0.14 gm/dL Hgb (A) or 100 µM heme (B). In (A), note that that the time-dependent release of TNFa induced by Hgb is inhibited by the TLR4 antagonist, VIPER. In (B), CXCL2 release appears to reach a plateau at 24 hrs, corresponding to the peak of neutrophil invasion of the CNS after ICH.

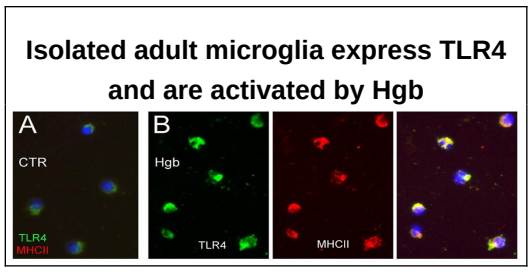


**Fig. 3.** Quiescent microglia were isolated from adult rat brain using discontinuous Percoll gradients. Cells were plated in Serine/Glycine deficient media, which induces a resting phenotype. Resting (A) and Hgb (0.14 gm/dL, 24 hr) activated (B) microglia labeled for TLR4 (green), and MHC-II (red); (nuclei, DAPI, blue). Note the marked upregulation of TLR4 (low levels in resting microglia, A) and of MHC-II(not present in resting microglia, A), a key identifier of the M1 microglial phenotype.

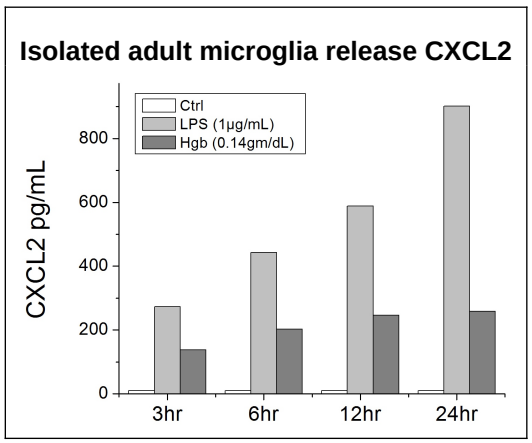


**Fig. 4.** ELISA for CXCL2 detected in the media of isolated adult microglia exposed to Hgb or LPS.

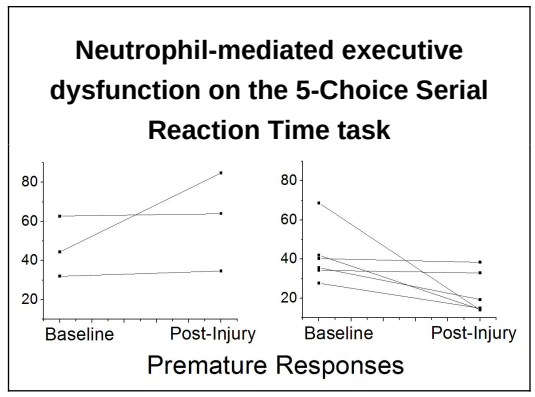


**Fig. 5.** 10µL CXCL2 (0.1 µg/µL) was injected into the VS of rats (N=9). Damage to the VS can lead to increased or decreased premature responses. On average, injury altered behavior by 38%.

## Discussion

This is the first report, to our knowledge, demonstrating that isolated adult microglia release CXCL2 in response to Hgb. Neutrophils respond to CXCL chemokines, invade the CNS, and can cause secondary injury. Our preliminary data suggest that neutrophil recruitment into the CNS by CXCL2 is sufficient to cause executive dysfunction. Microglial release of CXCLs in response to hemorrhage may be an important mechanism of neutrophil recruitment after ICH, and thus presents an opportunity for novel therapeutic interventions.