ImmunoTools *special* Award 2014

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MPO-chlorinated lipids induce ER stress in Brain microvascular endothelial cells

Most regions of the brain operate within a well-controlled environment. There, brain microvascular endothelial cells form the morphological basis of the blood brain barrier by formation of tight junctions (Hawkins et al, 2005), in order to prevent uncontrolled paracellular transport of nutrients and cells (Tsukita et al, 2001). Under inflammatory conditions the function of the blood brain barrier is compromised and can aggravate neuronal dysfunction. (Coisne et al, 2011)

During activation of phagocytes the Myeloperoxidase (MPO) - H_2O_2 – Cl^- system generates a variety of reactive oxidizing agents including hypochlorous acid (HOCl). Our group could recently demonstrate that MPO-positive neutrophils accumulate at the endothelium as shown by immunohistochemistry of sagittal cryosection of brain tissue from mice that received a single systemic LPS injection (Uellen et al, 2013). Under the presence of physiological chloride concentrations MPO which is considered as disease amplifying enzyme in neurodegenerative disorders uses H_2O_2 to generate the potent oxidant HOCl. Lipids are major targets of reactive chlorinating agents released by activated immunocells. HOCl has the ability to target primary amines, alkenes and vinyl ethers of lipids. Plasmologens, ether phospholipids are highly abundant in the brain and take a central role in proper brain function. The HOCl oxidation products derived from plasmologens are 2-chlorohexadecanal, alpha chlorofatty aldehyde and lysophospholipid. 2-Chlorohexadecanal can be metabolized to 2-Chlorohexadecanoicacid (2-ClHA) and 2-Chlorohexadecanol (2-ClHOH) by neutrophils and endothelial cells.

Chlorinated lipids are potent modulators of BMVEC barrier function and induce mitochondrial dysfunction, ATP depletion, apoptosis and trigger the formation of reactive oxygen species.

The endoplasmic reticulum (ER) is a essential organelle for synthesis folding and processing of transmembrane and secretory proteins. Physiological and pathological stimuli can disrupt ER homeostasis resulting in accumulation of unfolded and misfolded proteins, a condition known as ER stress. ER stress activates a complex signaling network referred as the unfolded protein response (UPR). However, if the UPR fails to re-establish the ER function ER stress leads to cell dysfunction and cell death. The UPR is initiated by three ER transmembrane proteins, the inositol requiring enzyme 1(IRE-1), the PKR-ER like kinase (PERK) and the activating transcription factor 6(ATF6). Under ER stress conditions PERK and IRE-1 are activated by formation of a dimer and autophosphorylation. The inactive form of ATF-6 is transported to the golgi organelle and is activated by two-step cleavage mediated by S1P and S2P proteinases (H. Yoshida et al, 2007; Adachi et al, 2008). PERK phosphorylates eIF-2α for attenuation of protein translation to counteract against accumulation of misfolded and unfolded proteins within the ER (Gotoh et al, 2006). Under severe and prolonged ER stress conditions UPR activation results in apoptosis to remove damaged cells (Gotoh et al, 2006; Oyadomari et al, 2004).

The IRE1α-TRAF2-IKK complex induces degradation of IκBα, activation of the inflammatory master regulator NF-κB, and the transcription of inflammatory genes such as TNF-α, TGF-β, IL-2, IL-6, and IL-8 (Schwabe et al, 2005; Gargalovic et al, 2006; Han et al, 2006). Activated ER stress sensor Ire1 activates JNK/p38 MAP kinase through the Ire1-TRAF2-ASK1 pathway (Homma et al, 2009). Moreover, Park et al. reported that the CHOP is involved in regulating the expression of proinflammatory cytokine IL-8 through activation of NF-κB (Park et al, 2010).

Based on results from ongoing work we propose that MPO-derived chlorinated lipids are potent activators of endoplasmic reticulum stress in the human brain microvascular endothelial cell line hCMEC/D3 leading to inflammation and apoptosis.

We were already capable to show that 2-ClHDA and its metabolites initiate the phosphorylation of eIF2-α and the expression of CHOP, a crucial event in the PERK mediated UPR activation.

Using the human cell line hCMEC/D3 as a model of the blood brain barrier we would like to examine the induction of apoptosis in response to 2-ClHDA, 2-ClHA and 2-ClHOH treatment using the ImmunoTools anti-human Annexin V antibody for flow cytometry.

Furthermore we want to elucidate whether chlorinated lipids induce the activation of proinflammatory cytokines in response to chlorinated lipids by ELISA using the ImmunoTools ELISA kits and if treatment of hCMEC/D3 cells with recombinant cytokines from ImmunoTools modulate blood brain barrier permeability.

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FITC - conjugated Annexin V,

human ELISA-set for 96 wells: human IFN-gamma, human IL-6, human IL-8, human IL-10, human IL-12p40 total (detect IL-23 as well), human TNF-a (each 3 reagents),

recombinant human cytokines: rh IL-6, rh IL-8, rh TNFα,

mouse IL-6 ELISA-set for 96 wells, (each 3 reagents),

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