Diabetic Complications Consortium

Application Title: Chromatin protein HMGB1 triggers endothelial dysfunction in diabetes via TLR4

Principal Investigator: Webb, R. Clinton

1. Project Accomplishments:

Endothelial dysfunction is one of the most important mechanisms mediating the major diabetic complications. Several factors contributing to endothelial dysfunction during diabetes (reduced NO bioavailability, increased production of reactive oxygen species) are intimately linked to vascular inflammation. These factors ultimately mediate increased endothelial cell apoptosis and reduced endothelium-dependent vasodilation and contribute to macro- and microangiopathy. Vascular inflammation is thus at the core of both providing understanding for the pathogenesis of vascular complications of diabetes and of devising novel therapeutic approaches to address them. The chromatin protein HMGB1 is thought to be increased in conditions characterized by low grade sterile inflammation such as diabetes, whether through accidental release from necrotic cellular injuries or through active secretion. Activation of TLR4 by HMGB1 leads to increased NFκB siganling, reactive oxygen species and cytokine production in immune cells. In this project, we examine the role of endothelial TLR4 activation by HMGB1 in diabetic endothelial dysfunction.

2. Specific Aims and Results:

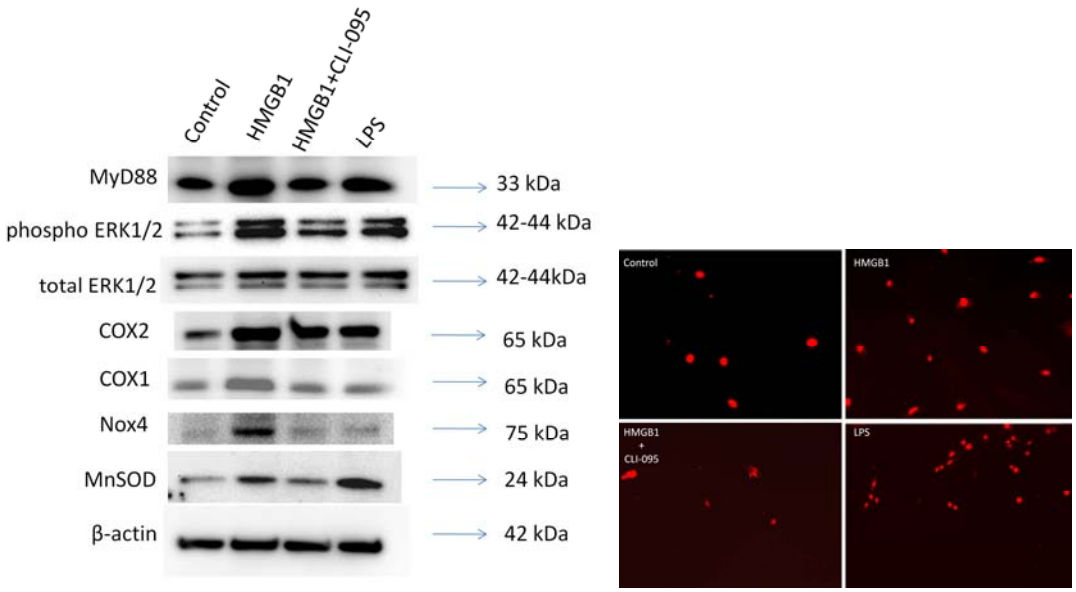
Specific aim 1 tested the hypothesis that HMGB1 activates TLR4 signaling in EC leading to increased ROS generation, decreased NO bioavailability and NFκB activation resulting in EC apoptosis and inflammation.

Specific aim 2 tested the hypothesis that HMGB1 induces impaired endothelium-dependent vasodilatation in diabetes through mechanisms that involve TLR4.

We addressed our hypotheses *in vitro* using a cell culture model of human aortic endothelial cells (HAEC) and *in vivo* using a TLR4 knockout (strain B6.B10ScN-Tlr4lps-del/Jth, #007227 Jackson) model of streptozotocin-induced diabetes (low dose protocol - 50 mg/kg i.p. daily for 5 consecutive days).

Briefly, exposure of cultured human aortic endothelial cells to recombinant human HMGB1 time and concentration-dependently induced activation of TLR4 as evidenced by expression of its downstream partner MyD88. HMGB1 also induced increases in phosphorylation of the MAPK pathway (ERK) and nuclear translocation of NFκB, which likely mediated increased expression of cyclooxygenase (both isoforms). We observed an increase in the expression of Nox4 subunit of NADPH oxidase and increased reactive oxygen species production, and as a result upregulation of anti-oxidant pathways and expression of the mitochondrial isoform of superoxide dismutase MnSOD. These effects mimicked the TLR4 activation effects of lipopolysaccharide (LPS), which was used in most experiments as a positive control. Importantly, all these effects were inhibited or reversed by pretreatment of HAEC with the TLR4 antagonist CLI-095.

We observed that diabetes induction by streptozotocin leads to increased protein expression of HMGB1 in plasma. Genetic deletion of TLR4 does not protect mice from diabetes induction by streptozotocin, suggesting that TLR4 is not involved in the pathogenesis of the insulin deficiency observed in this model. In vivo treatment of diabetic mice with TLR4 antagonist CLI-095 has partially restored the reduced endothelium-dependent vasorelaxation, and we are currently testing whether genetic deletion of TLR4 would have the same beneficial effect.



Protein expression measured by western blotting (left) and reactive oxygen species measured by DHE fluorescence (right) after 24h exposure of HAEC to vehicle (control), LPS (100 ng/ml, positive control) or recombinant HMGB1 (100 ng/ml, TLR4 agonist) alone or in the presence of CLI-095 (1 μM, TLR4 antagonist).

3. Publications:

Szasz T, Ogbi 2, Webb RC. Activation of Toll-like receptor 4 (TLR4) by high mobility group box 1 (HMGB1) leads to oxidative stress and inflammation in human aortic endothelial cells. (abstract submission, Experimental Biology 2016).