

Epigenetically Altered TLR4 Expression May Contribute to Increased Inflammation and Impaired Wound Healing in a Murine Model of Diabetes

Andrew Kimball, Amrita Joshi, Matthew Schaller, Beau Carson, Kanakadurga Singer, Jennifer Bermick, Shreyas Ramani, Peter Henke, Steven Kunkel, Katherine Gallagher, Univ of Michigan, Ann Arbor, MI

BACKGROUND: Wound healing is impaired in diabetes due to dysregulated inflammation. Previous studies by our group and others have demonstrated that inflammatory mediators are significantly increased in diabetic wounds, however, the mechanisms behind this hyper-inflammatory response remain largely unknown. As there is known to be high levels of endotoxin in type 2 diabetic (T2D) wounds, we aimed to assess the role of high fat diet (HFD) on Toll-Like Receptor 4 (TLR4) expression. We hypothesized that HFD/T2D would alter histone methylation, leading to increased receptor expression and enhanced inflammation.

METHODS: C57/BL6 mice were subjected to either normal or HFD for 12-16 weeks to induce the diet-induced obese (DIO) model of physiologic T2D. 4mm hindlimb wounds were created and healing was monitored daily using NIH ImageJ software. Bone marrow-derived macrophages (BMDM) were cultured and TLR4 expression measured by qPCR. Myeloid TLR4 protein expression was assessed in peripheral blood and wound cell isolates by analytical flow cytometry. Macrophages (NK1.1-/Ly6G-/CD11b+) were isolated using magnetic sorting and inflammatory cytokine expression determined with qPCR and bioplex. Chromatin Immunoprecipitation (ChIP) analysis at the NF- κ B binding site on the TLR4 promoter was performed using antibodies to trimethylated Histone 3 Lysine 4 (H3K4me3). (N= \sim 5 mice per group for all studies).

RESULTS: DIO mice demonstrated significantly delayed wound healing as compared to littermate controls and BMDM isolated from these mice revealed increased TLR4 gene expression. Correspondingly, peripheral blood and wound monocyte/macrophages showed increased TLR4 receptor levels by flow cytometry. ChIP analysis at the TLR4 promoter revealed significantly increased H3K4me3, a gene activating mark, in the HFD BMDM. Finally, macrophage wound isolates from DIO mice had elevated levels of inflammatory gene expression and cytokines.

CONCLUSION: Enhanced H3K4me3 at the TLR4 promoter in diabetic macrophages may increase receptor expression and contribute to the hyper-inflammatory response seen in T2D. Further mechanistic studies are needed in order to examine the role of TLR4 in wound healing.

A. Kimball: None. **A. Joshi:** None. **M. Schaller:** None. **B. Carson:** None. **K. Singer:** None. **J. Bermick:** None. **S. Ramani:** None. **P. Henke:** None. **S. Kunkel:** None. **K. Gallagher:** None.