

0518 Engagement of RAGE in Diabetic Neutrophils Contributes to Cell Priming and Altered Neutrophil Response

M. KARIMA¹, **A. KANTARCI**¹, **H. HASTURK**¹, **B.-H. NAM**², **A.O. MALABANAN**³, **P.C. TRACKMAN**¹, and **T.E. VAN DYKE**^{1, 1} Boston University Goldman School of Dental Medicine, MA, USA, ² Boston University, Department of Mathematics and Statistics, MA, USA, ³ Boston University, Department of Medicine, MA, USA

Advanced glycation end products (AGE's) accumulate in diabetic plasma and tissue due to hyperglycemia. AGE's are heterogeneous molecules that include carboxymethyllysine (CML), pyralline, and pentosidine. AGE-mediated events important in pathogenesis of diabetic complications. Neutrophil function is altered in diabetics, and the presence of periodontal disease aggravates the neutrophil response. In addition, engagement of receptor for AGE (RAGE) by AGE on hematopoietic cells is critical in inflammation.

Objective: Aim of this study is to determine whether AGEs stimulate neutrophil response in diabetics with or without periodontitis, and whether this response is mediated by RAGE.

Methods: RAGE on neutrophils from healthy and DM subjects was detected by Western blotting of cell extracts. CML-albumin was made by chemical modification of bovine serum albumin, and endotoxin was removed by affinity chromatography. Superoxide production in the presence and absence of function blocking RAGE antibody was compared in neutrophils primed with CML-albumin and control unmodified albumin.

Results: We identified the presence of RAGE on both normal and DM neutrophils by Western blots of cell extracts, and specificity of the antibody was confirmed in competition studies. Superoxide production from diabetics and healthy controls was blocked by the same RAGE antibody upon stimulation of the cells with CML-albumin. Unmodified control albumin stimulated little superoxide production in neutrophils as expected. CML-albumin induced a dose-dependent increase in neutrophil superoxide production obtained from healthy and diabetic subjects. Superoxide was two-fold higher in unstimulated diabetic neutrophils (n=20; p<0.001) and when cells were incubated with CML as a priming agent, followed by stimulation with a second agonist (fMLP or PMA), superoxide production was rapid and 3.5-fold higher in diabetics (p<0.001).

Conclusions: These findings suggest that RAGE plays an important role in initiating cellular signaling that lead to excessive neutrophil function (priming) characterized by elevated superoxide production. Supported by USPHS Grants DE13191, DE14478.