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Allen A[1], Shobeiri S A[1], Nihira M[1], Quiroz L[1]
1. *The University of Oklahoma HSC*

EXPRESSION OF VEGF AND PEDF IN THE DIABETIC MOUSE BLADDER: MARKERS OF AN INFLAMMATORY PROCESS

Hypothesis / aims of study

Vascular endothelium growth factor (VEGF) and pigment epithelium-derived factor (PEDF) maintain significantly inter-related roles in chronic inflammatory conditions such as diabetes. These two proteins create a balance for the maintenance of vascular and tissue health. In disease processes, however, this balance can become disrupted leading to organ damage and failure. The objective of this study was to evaluate the expression of these two inflammatory markers in diabetic and non-diabetic mouse bladders. The hypothesis was that VEGF and PEDF will display opposing protein expression in the diabetic mouse bladder compared to the non-diabetic mouse bladder.

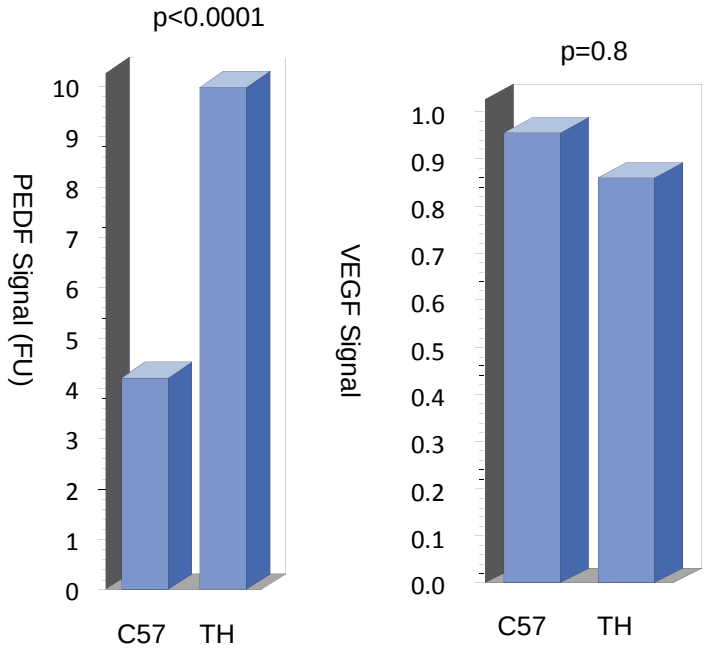
Study design, materials and methods

Three TallyHo (TH) diabetic and six C57BL/6 (C57) non-diabetic mice were sacrificed at 12 weeks of age. The bladders were immediately harvested and embedded in freezing medium. Cryostat sections (10 m) underwent immunohistochemical staining with the following primary antibodies: mouse monoclonal antibody to VEGF (1:50 dilution), and rabbit polyclonal antibody to PEDF (1:250 dilution). Slides were incubated with the appropriate secondary antibodies at a 1:400 dilution, and then counterstained with DAPI. Controls included an equal number of bladder sections stained with the omission of the primary antibody. With the use of ImageJ freeware, the intensity of fluorescent signal was quantitatively determined in each of four quadrants of a digital image for each bladder section (24 total TH and 48 total C57 images for each antibody) by normalizing with background signal value.

Results

All signal intensity values were averaged separately for the TH and C57 strains and then compared. Statistical analysis was performed with SAS v.9.2. P-values <0.05 were considered statistically significant. The PEDF signal intensity (in fluorescence units, FU) was significantly higher in TH bladders, with an average signal value of 9.98 FU (SD±3.9), compared to C57 bladders whose average signal value was 4.21 FU (SD±4.0), ($p<0.0001$). The expression of VEGF was not significantly different between the mouse strains, with an average value of 0.95 FU (SD±2.2) in C57 bladders, and 0.86 FU (SD±1.1) in TH bladders ($p=0.8$).

Figure 1. PEDF and VEGF expression in C57 and TH mouse bladders



Interpretation of results

Diabetic mouse bladders demonstrate a significant increase in PEDF expression as compared to non-diabetic mice, whereas VEGF levels remained approximately equal. This is likely evidence of a diabetes-induced manifestation in the mouse bladder of the inflammatory process that occurs in the presence of poor glycemic control. Tissues, including the bladder, typically respond to injury, such as diabetic-related injury, with an acute inflammatory response that may be followed by chronic inflammation and repair [1]. Under high glucose conditions, several studies have investigated the interplay between the inflammatory mediators, PEDF and VEGF, in the development of diabetic pathology, and have found opposing expression.

Concluding message

The elevated PEDF levels indicate the presence of an ongoing inflammatory process, and it is likely that the physiologic repair mechanisms occurring in these mouse bladders may also occur in the human bladder. These findings may be a step towards understanding the pathophysiology of diabetic bladder dysfunction.

References

1. Saban, M.R., et al., Gene expression profiling of mouse bladder inflammatory responses to LPS, substance P, and antigen-stimulation. American Journal of Pathology, 2002. 160(6): p. 2095-110.

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Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
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