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Authors: J. Lowe, A. Banerjee, B. Siegmund, G. Fantussi, F. Daneshgari
Institution: University of Colorado Health Sciences Center
Title: TUMOR NECROSIS FACTOR-ALPHA (TNF-a) IS NOT EXPRESSED IN THE BLADDER SMOOTH MUSCLE CELLS OF DIABETIC RAT.

Aims Of Study:

The pathophysiology of diabetic cystopathy is poorly understood. Hyperglycemia causes an increased expression of Nuclear Factor- kappa B (NF-□B) in human vascular smooth muscle cells, possibly leading to impaired contractility. This function of NF-□B is thought to be due to its induction of TNF-□which initially increases, and then inhibits, the activity of a number of key enzymes involved in energy metabolism thus leading to cell dysfunction. We sought to determine the expression of NF-□B and TNF-□ in the diabetic bladder.

Methods:

Our laboratory has recently described the use of a transgenic rat model of DM for studies of lower urinary tract dysfunction. The bladders of these rats were harvested, weighed, and fixed in 10% formalin, OCT compound or snap frozen. The formalin fixed tissues were sectioned and stained using H&E for light microscopy (LM) examination. The OCT fixed tissues were incubated with rabbit polyclonal primary antibodies against NF-□B and TNF-□ and secondary goat anti-rabbit antibodies conjugated to Cy-3 (Santa Cruz Labs, CA). The sections were further stained to visualize membrane glycoproteins (Wheat Germ Agglutination conjugated to Alexa 488) and to visualize nuclei (Blue: Bis-benzamide). Triple stained sections were then imaged using confocal microscopy. Snap frozen samples were homogenized and centrifuged for Enzyme Linked Immumosorbent Assay (ELISA) for TNF-

Results:

Four DM and four control animals were used. The LM examination showed a) increased interstitial edema; b) separation of detrusor smooth muscle cells (DSMC); c) increased collagen formation in the interstitium; and d) hypertrophy of DSMC. Immunohistochemical studies using Confocal microscopy showed absence of TNF-□throughout the DSMC and urothelium. ELISA also showed absence of TNF-□in the homogenized bladder tissue. In contrast, NF-□B was detected in the bladder and found to be localized to the DSMC nuclei.

Conclusions:

In contrast to a previously described relationship between TNF-□ and NF-□B in smooth muscle cell dysfunction, the results of our study indicate an absence of TNF-□ protein in the DM bladder, despite ample nuclear NF-□B staining in the DSMC. This finding may indicate that the signal transduction pathways involved in diabetic cystopathy is more complex than that described in other smooth muscle cells and warrants further investigation.

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