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ADENOVIRUS-MEDIATED INHIBITION OF NF-□B ACTIVATION SUPPRESSES INDUCTION OF PROINFLAMMATORY CYTOKINES EXPRESSION IN BLADDER EPITHELIAL CELLS: POSSIBLE ROLE IN INTERSTITIAL CYSTITIS

Aims of Study

A number of factors have been shown to activate NF-□B, and several signal transduction pathways may be involved; all these stimuli act by means of protein kinases that phosphorylate I□B. These include pathogens, cytokines, growth factors, activators of protein kinase C, viruses, oxidants, and stress[1].

Although the nuclear transcription factor NF-□B has been implicated in many inflammatory diseases, the downstream mechanism(s) by which it can mediate it effects are still fragmentary. We have previously reported, for the first time, activation of the nuclear factor NF-□B in bladder biopsies of IC patients, predominantly in the cells of the urothelium and submucosal layer[2]. In this study, we examined the role of this nuclear factor on induction of proinflammatory cytokines in the bladder urothelial T24 cell line and further examined their corresponding secretory levels in the urine of IC patients.

Methods

T24 cells were were transduced (20 MOI) for 24 hr with a dominant-negative super-repressor I□B mutant (pAxCAmI□B-M) or wild-type (pAxCA) adenoviral constructs in presence or absence of stimulants. Transduction efficiency was monitored *in situ* using a reporter Adv-gal construct, whereas the efficacy of inhibition of NF-□B activation was evaluated by gel mobility shift assay. Urine samples were collected from IC patients (*n=90*) and healthy controls and appropriately stored for subsequent analysis. All patients were clinically diagnosed as having interstitial cystitis and were selected according to NIDDK criteria. Expression profile analysis of proinflammatory cytokines was measured in cells and urine using RT-PCR and ELISA, respectively

Results

Activation of NF-□B by rhTNF- was associated with 27, 8, 10 and 7-fold increase in the TNF-, IL-1, IL-6 and IL-8 transcripts, respectively. In contrast, abrogation of the TNF--induced cytokine gene expression by an adenovirus super-repressor I□B mutant vector suggested that these effects were NF-□B-dependent. Interestingly, the upregulation of gene transcripts correlates with increased protein levels of NF-□B-activating and/or regulated factors, including IL-1 (2 fold), IL-2 (7 fold), IL-6 (6 fold), TNF- (3 fold), and IFN- (27 fold), in the urine of IC patients in comparison to healthy controls.

	IL-1β	IL-2	IL-4	IL-6	IL-8	IL-10	TNF-α	IFN-γ
IC Patients	436 ± 65.3	2.79 ± 0.9	239.6 ±109	69 ± 23	139 ± 17.2	20.0 ± 3.5	364 ± 63.3	407.1 ±110.3
Controls	191.5 ± 49.6	0.40 ± 0.07	93.7 ± 6.8	11.6 ± 1.9	17.9 ± 10.5	23.6 ± 3.4	116.1 ± 66.7	27.3 ± 8.1

Table 1: Detection of Inflammatory and Immune Response Factors in Urine of IC Patients and Healthy Controls. Values are expressed in pg/ml; M±SE

Conclusions

The fact that these factors are capable of inducing activation of urothelial cells' NF-□B suggests a pivotal role for this nuclear transcription factor in the pathophysiology of the disease, possibly by inducing aberrant immune and inflammatory responses within the bladder of IC patients.

References

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