

Effect of Cigarette Smoke-Induced Oxidative Stress on Cytokine Production in Transformed Lung Epithelial Cells



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INTRODUCTION

- 20.8% of Americans (45.3 million) currently smoke cigarettes.
- Cigarette Smoke is the main etiological factor in the development of COPD
- Cigarettes are a mixture more than 4700 chemical compounds including reactive oxygen and nitrogen species.
- Past studies have shown cigarette smoke to activate complex receptor-mediated signaling pathways that impair normal lung function, such as NF- κ B.
- The most obvious response to oxidant-mediated lung injury is the generation of pro-inflammatory cytokines.
- Research on pro-inflammatory gene transcription is needed to develop preventive drugs in an effort to prevent and inhibit disease.

**American Cancer Association
Lung Cancer in the United States
2008 Estimate**

New Cases: 215,020

Deaths per Year: 161,840

Five year survival rate for all stages combined: 15%



AIM

- Determine the activation of RelA/p65 subunits of NF- κ B and nuclear translocation in response to cigarette smoke extract.
- Determine the time and dose-dependent induction of IL-8 with cigarette smoke extract both in transformed bronchial epithelial cells (Beas-2B) and cancer bronchial epithelial cells (H292).

METHODS

Cell culture

Transformed bronchial epithelial cells (Beas-2B):

BEAS-2B cells are derived from transforming primary bronchial epithelial cells with adenovirus simian vacuolating virus (SV-40). Cells were grown in Dulbecco's modified Eagle's medium-Ham's F12 50:50 mixture (DMEM-F12) supplemented with 5% FBS, 15 mM HEPES, 100 mg/ml penicillin, and 100 units/ml streptomycin.

Human cancer bronchial epithelial cells (H292):

H292 cells are derived from a patient with bronchial mucocellular carcinoma. Cells were grown in RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, 100 μ g/ml penicillin, and 100 U/ml streptomycin in humidified atmosphere under 5% CO₂ at 37°C.

Preparation of cigarette smoke extract (CSE)

CSE (10%) was prepared by bubbling smoke from one cigarette into 10 ml of culture media at a rate of 1 cigarette/2 min.

Treatments

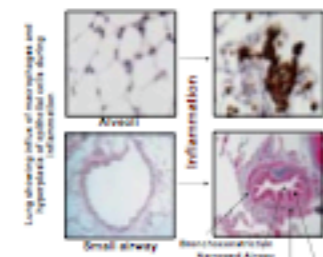
Beas-2B and H292 cells were seeded at a density of 1.0 x 10⁶ cells in 6-well plates in a total volume of 2 ml DMEM-F12 containing 1% serum and RPMI 1640 containing 0.5% FBS respectively. Cells were treated with CSE (0.5 and 1.0 %) for 4 and 24 hours respectively.

ELISA:

The culture medium/supernatant was collected after treatment. The levels of IL-8 cytokine in the supernatant were determined by sandwich ELISA, using the dual antibody kits according to the manufacturer's instructions.

Immunocytochemical (ICC) Analysis of NF- κ B RelA/p65 localization

Activation of NF- κ B was assessed by immunocytochemical localization of RelA/p65 subunit of NF- κ B. Cells were then treated with CSE (1.0%), TNF- α (10 ng/ml) and LPS (1 μ g/ml) as a positive control for 20 min and 2hrs.

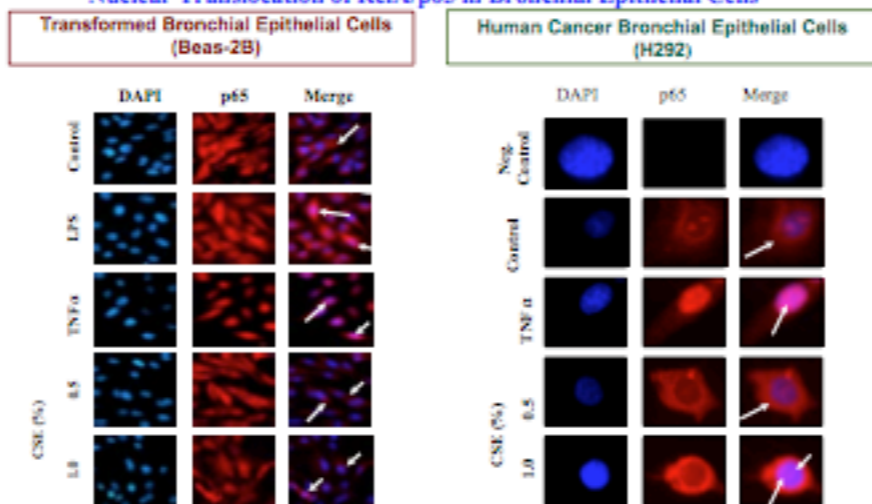


HYPOTHESIS

Cigarette smoke differentially induces pro-inflammatory gene transcription through the NF- κ B pathway in transformed cancer and noncancerous lung epithelial cells.

RESULTS

Nuclear Translocation of RelA/p65 in Bronchial Epithelial Cells



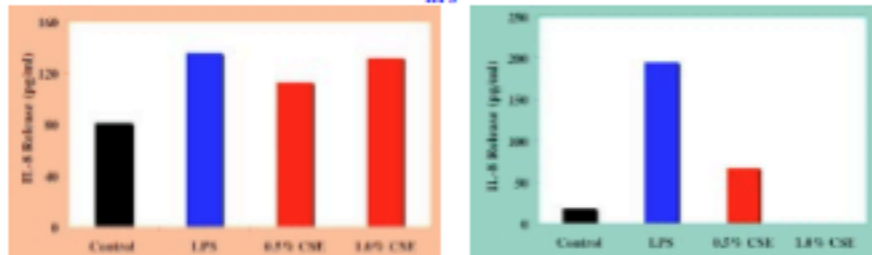
Note: DAPI = nucleus staining. Arrows denote p65

RESULTS

Effect of Cigarette Smoke Extract on IL-8 Release in Bronchial Epithelial Cells at 4 hrs



Effect of Cigarette Smoke Extract on IL-8 Release in Bronchial Epithelial Cells at 24 hrs



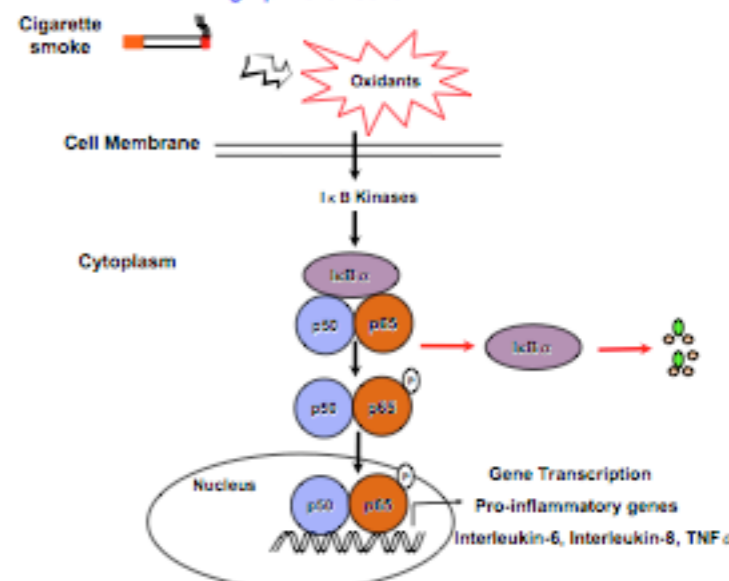
SUMMARY

• Various cell types showed differential effect to cigarette smoke extract and LPS assessed by cytokine release.

• TNF- α and LPS was more potent at inducing nuclear RelA/p65 translocation in lung epithelial cells.

• RelA/p65 nuclear translocation correlated with cytokine release.

Model for Mechanism of NF- κ B Activation Leading to Gene Transcription in Lung Epithelial Cells



Conclusion

Cigarette smoke induced oxidative stress leads to RelA/p65 nuclear translocation and consequently increased pro-inflammatory cytokine release.

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