

Diesel exhaust particles affect expression and arrangement of the tight junction protein occludin in lung cells *in vitro*

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The human airway epithelium serves as a structural and functional barrier against inhaled particulate antigen. Using an *in vitro* triple cell co-culture model of the epithelial airway barrier consisting of human epithelial cells, monocyte-derived macrophages and dendritic cells [1, 2], it was recently demonstrated that macrophages and dendritic cells create a transepithelial network between the epithelial cells to capture antigens without disrupting the epithelial tightness. The

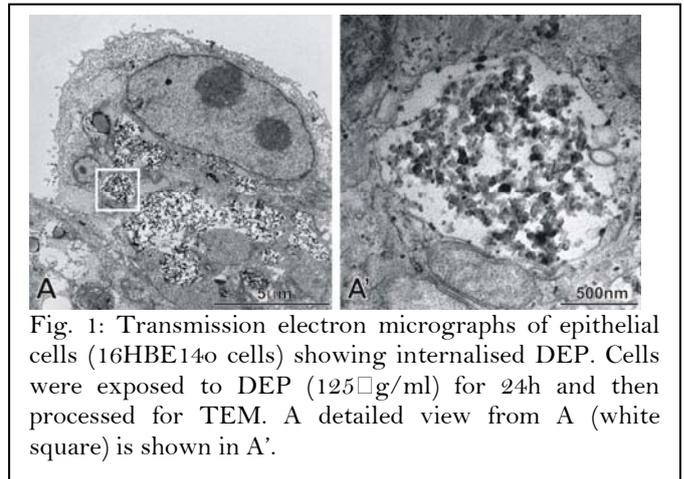


Fig. 1: Transmission electron micrographs of epithelial cells (16HBE14o cells) showing internalised DEP. Cells were exposed to DEP (125 μg/ml) for 24h and then processed for TEM. A detailed view from A (white square) is shown in A'.

expression of the different tight junction proteins in macrophages and dendritic cells has been demonstrated [3].

Immunofluorescent methods combined with laser scanning microscopy and quantitative real-time PCR were used to investigate if exposure to diesel exhaust particles (DEP) at different concentrations (0.5, 5, 50, 125 μg/ml) for 24h is cytotoxic (LDH assay) and if DEP can affect the expression of the tight junction mRNA/protein of occludin in all three cell types. The spatial location of occludin in the different cell types was investigated by laser scanning microscopy and the tight junction arrangement by transmission electron microscopy.

Using transmission electron microscopy DEP was found in membrane bound vesicles inside all cell types (Fig. 1). No cytotoxicity was measured in all cell cultures exposed to the different DEP concentrations. Only the highest dose of DEP (125 μg/ml) appeared to reduce the occludin mRNA expression in the immune cells but not in epithelial cells, although the occludin arrangement in the latter cell type was disrupted. The transepithelial electrical resistance was reduced in epithelial cell mono-cultures but not in the triple cell co-cultures after exposure to high DEP concentration.

We conclude that high concentrations of DEP (125 μg/ml) may affect the tight junction occludin mRNA in the immune cells and that those cells play an important role maintaining the epithelial integrity after exposure to particulate antigens in lung cells.

This work is supported by the Swiss National and the German Research Foundation.

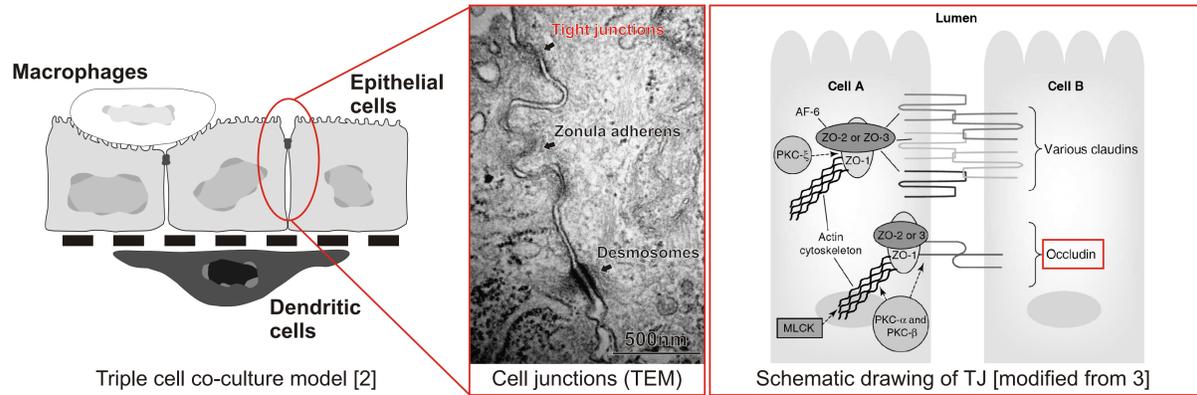
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[2] Blank et al. *Am J Respir Cell Mol Biol* 2007;36:669-77.

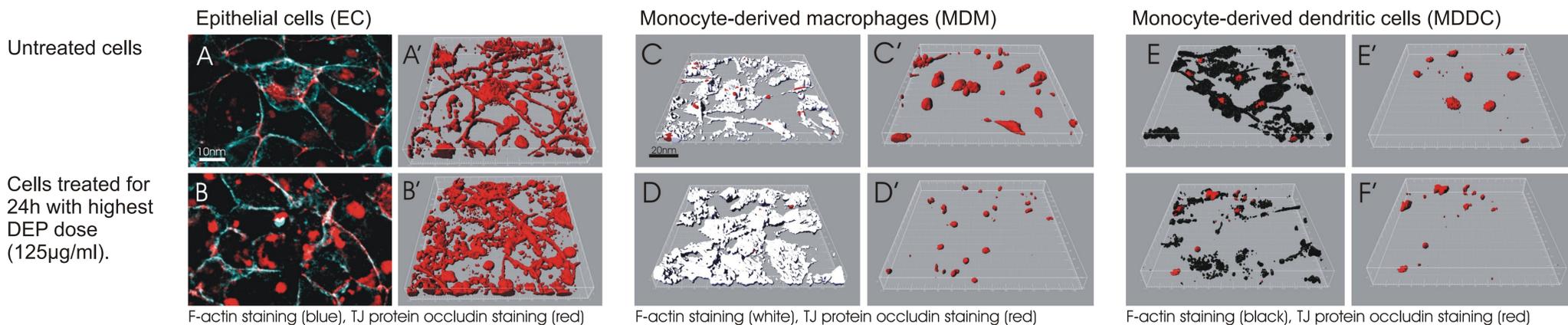
[3] Blank et al. *submitted 2009*

Introduction & Hypothesis

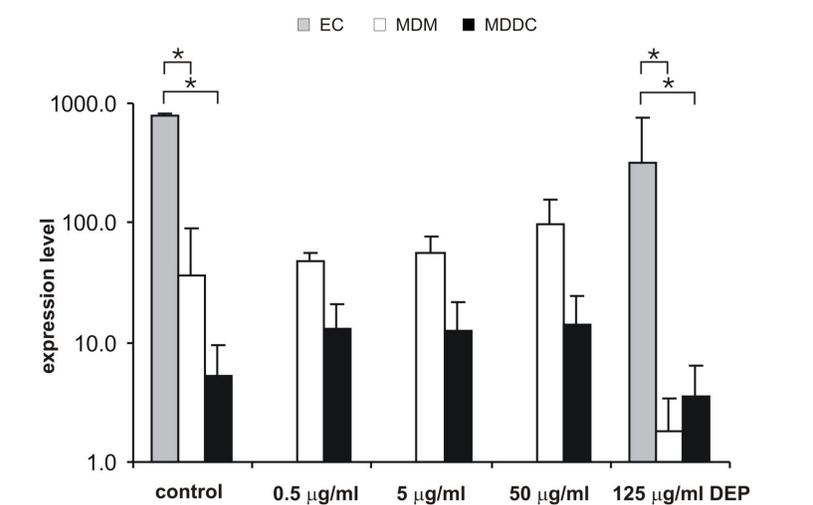
The airway epithelium acts as a barrier against inhaled particles and is sealed by **tight junctions (TJ)**. Macrophages on top and dendritic cells at base of the epithelial cells (EC) create a transepithelial network by forming processes between EC to capture particulate antigens without affecting the epithelial integrity [1]. Diesel exhaust particles (DEP) are known to induce adverse effects in the human body. In the present study we investigated if exposure to DEP (0.5, 5, 50, 125 µg/ml) for 24h modulate the expression of the TJ mRNA/protein occludin in EC, monocyte-derived macrophages (MDM) and monocyte-derived dendritic cells (MDDC). Under the same exposure condition epithelial mono-cultures were compared to the triple cell co-culture in terms of the functional epithelial integrity, inflammatory response and cell death.



TJ occludin protein by immunostaining in monocultures



TJ occludin mRNA expression in monocultures



EC (white columns) have a significantly higher expression of occludin mRNA. MDM (grey columns) and MDDC (black columns) express occludin mRNA but there is no difference between both cell types. There is no significant dose-response relationship but it might be the highest dose of DEP (125 g/ml) reduce the expression after 24h of exposure. (n = 3-7)

Conclusions

Monocultures - We have demonstrated that even after exposure to a high dose of DEP (125µg/ml), the expression of occludin mRNA in EC monocultures remained unaffected, while TEER decreased significantly and the local distribution of the occludin protein changed to an irregular pattern. Both the protein and the mRNA levels of occludin in MDM and MDDC seem to be affected upon exposure to high DEP doses.

Triple co-cultures - In contrast, no change in TEER and unchanged TNFα levels were observed in the triple cell co-culture model. Further studies are required to understand the alterations of mRNA expression of the three cells types after stimulation or exposure to particles in the triple cell co-culture model and to elucidate the influence of the cell-cell interactions between the different cell types.

Literature

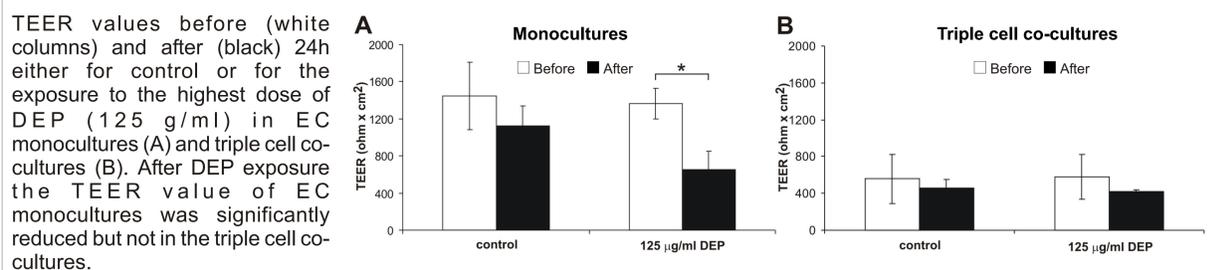
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Acknowledgments

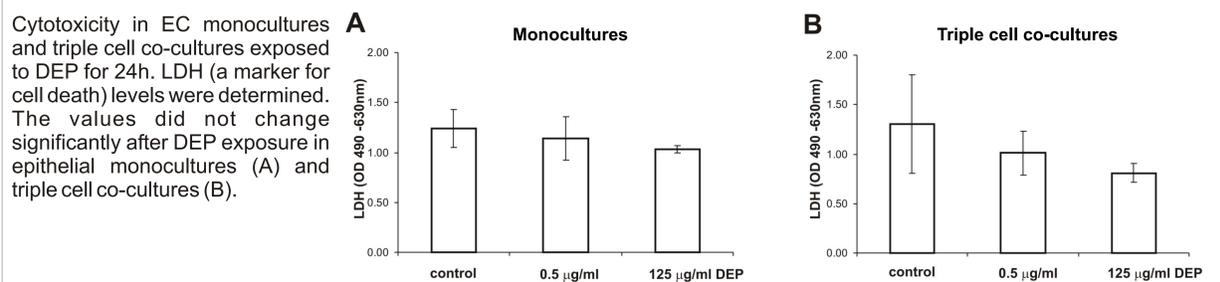
We thank **Barbara Tschirren, Andrea Stokes, Mohammed Ouanella** and **Barbara Krieger** for their excellent technical assistance. The work was supported by the Swiss National Sciences Foundation (SNF) and the German Research Foundation (DFG).

DEP effects on EC monocultures vs triple cell co-cultures

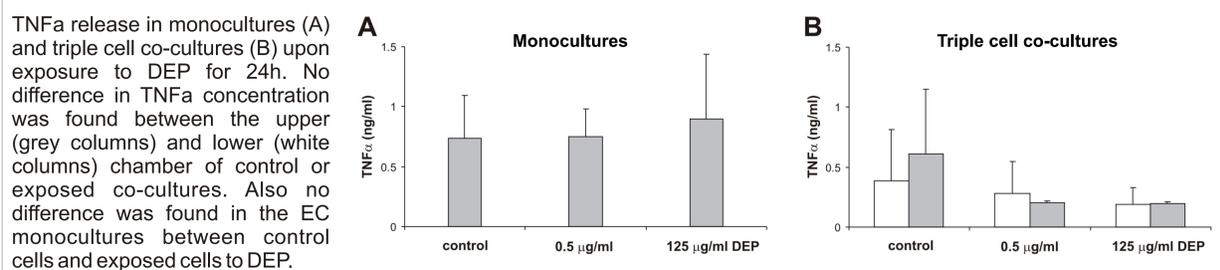
Transepithelial electrical resistance (TEER)



Lactate dehydrogenase release (LDH)



Tumor necrosis factor alpha (TNFα)



Methods

Cell Cultures

- The 16HBE14o EC line was used [3]; MDM, MDDC were derived from human blood monocytes [3]
- Co-cultures were produced as described in [3]; Different concentrations of DEP (SRM Nr. 2975, NIST) were exposed in suspension for 24h

Confocal Laser Scanning Microscopy (Zeiss 510 META)

- After fixation and staining cells were stained for F-actin and occludin
- For image processing and visualization IMARIS software (Bitplane AG, Zurich, Switzerland) was used

RNA isolation - Reverse Transcriptase - Real-time quantitative PCR

- Total mRNA was isolated from MDM, MDDC and EC using RNeasy mini kit (Qiagen) followed by reverse transcriptase and quantitative real-time PCR
- Beta-actin was used as housekeeping gene and the data were evaluated by the relative quantification method (2-delta CT) [4]

Cytotoxicity

- Lactate dehydrogenase in the supernatants was determined (Cytotoxicity detection assay, Roche, Roche Diagnostics, Rotkreuz, Switzerland)

TEER

- Mean of four measurements per insert were determined using Millicell-ERS