Analysis of tight junction protein expression in macrophages and dendritic cells after exposure to diesel exhaust particles and its functional aspects drawn from a cellular airway model

A.D. Lehmann, F. Blank, O. Baum, M. Wehrli, P. Gehr, and B. Rothen-Ruthishauser

The airway epithelium which acts as a barrier against inhaled particles is sealed by tight junctions (TJ). Macrophages on the top and dendritic cells at the base of the epithelial cells (EC) create a transepithelial network by forming processes between EC to capture particulate antigens without affecting the epithelial integrity. When particulate antigens, as for instance diesel exhausted particles (DEP), are deposited on airway surfaces they can easily pass through the plasma membrane to be incorporated into the epithelial cells or immune cells leading to adverse effects in the human body.
Using an *in vitro* triple cell co-culture model consisting of human EC, macrophages and dendritic cells, we could recently demonstrate the expression of TJ Protein occludin in EC and surprisingly also in the macrophages and dendritic cells in the co-cultures and respective mono-cultures by laser scanning microscopy (LSM).
The aim of this study was to quantify the mRNA expression of the TJ occludin and to investigate whether the exposure to inflammatory stimuli or DEP can modulate the mRNA expression of TJ proteins in the three cell types.
The mRNA expression of occludin in the mono-cultures was determined by quantitative real-time PCR after the exposition to LPS (lipopolysaccharides; an inflammatory stimulus) or to different concentration of DEP (0.5, 5, 50, 125µg/ml) for 24h. TJ-like complexes between macrophages, dendritic cells and EC in the *in vitro* model were analyzed using transmission electron microscopy (TEM).
TEM analysis suggested the formation of TJ-like complexes of macrophages and dendritic cells with the epithelium. Macrophages and dendritic cells expressed TJ mRNA, however, to a different extent. As expected, the mRNA levels of TJ were always lower than that of EC. Some mRNA expression patterns were modulated by exposure of the cells to LPS. First experiments give hints that occludin mRNA is increasing after exposure of the cells to the highest concentration of DEP.
We conclude that macrophages and dendritic cells constantly express the TJ protein occludin. The formation of TJ- like complexes between EC and macrophages or dendritic cells might contribute to the constant integrity of the lung epithelium during the uptake of inhaled particulate antigens in an interdigitating transepithelial network of macrophage and dendritic cell cytoplasmic processes. The analysis of the mRNA occludin in LPS and DEP-treated cells provide evidence that LPS as well as DEP are able to modify the human airway integrity by altering the expression of TJ mRNA.

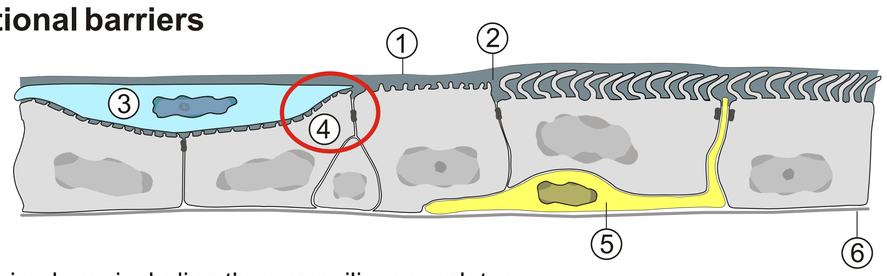
Analysis of tight junction protein expression in macrophages and dendritic cells after exposure to diesel exhaust particles and its functional aspects drawn from a cellular airway model

Andrea D. Lehmann, Fabian Blank, Oliver Baum, Peter Gehr, and Barbara M. Rothen-Rutishauser
Institute of Anatomy, University of Bern, Bern, Switzerland - mail to: andrea.lehmann@ana.unibe.ch

Introduction

The airway epithelium which acts as a barrier against inhaled particles is sealed by tight junctions (TJ). Macrophages on the top and dendritic cells at the base of the epithelial cells (EC) create a transepithelial network by forming processes between EC to capture particulate antigens without affecting the epithelial integrity [4]. When particulate antigens, as for instance diesel exhausted particles (DEP), are deposited on airway surfaces they can pass through the cell membrane to be incorporated into the Ecs or immune cells leading to adverse effects in the human body.

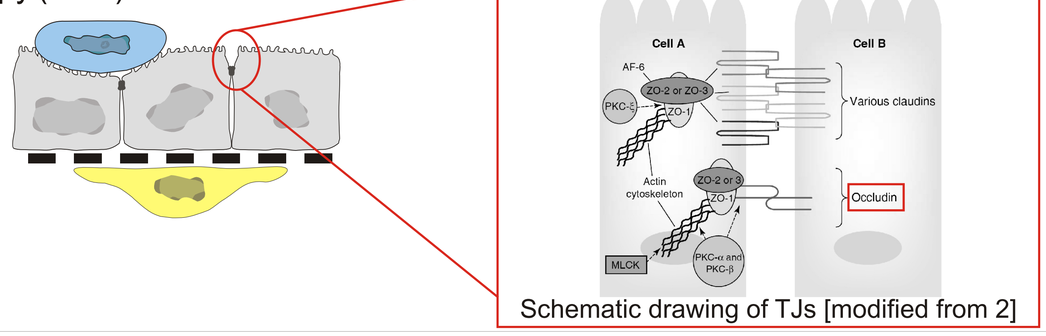
Structural and functional barriers



[1]

(1) the surfactant film
(2) the aqueous surface lining layer including the mucociliary escalator
(3) a population of macrophages (professional phagocytes) in the airways and in the alveoli
(4) an epithelium with tight junctions
(5) a population of dendritic cells (professional antigen presenting cells) in the airways and in the alveoli
(6) the basal lamina

Using an *in vitro* **triple cell co-culture model of the epithelial airway model** [2], we could recently demonstrate the expression of TJ Protein occludin in EC and also in monocyte-derived macrophages (MDM) and monocyte-derived dendritic cells (MDDC) in the co-cultures and respective mono-cultures by laser scanning microscopy (LSM).



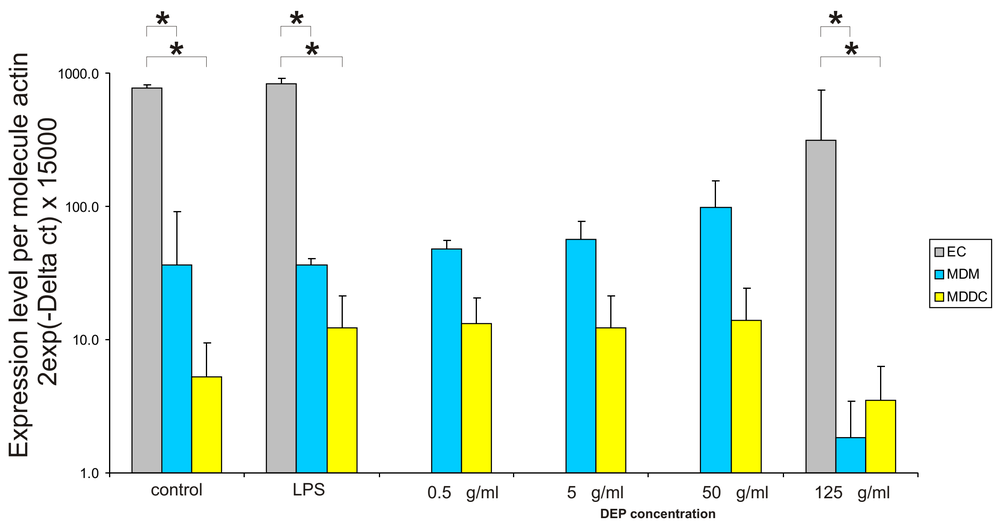
Schematic drawing of TJs [modified from 2]

Aim

The aim of this study was to quantify the TJ occludin mRNA expression and to investigate whether the exposure to inflammatory stimuli or DEP can modulate the mRNA expression of TJ proteins in monocultures of the three cell types.

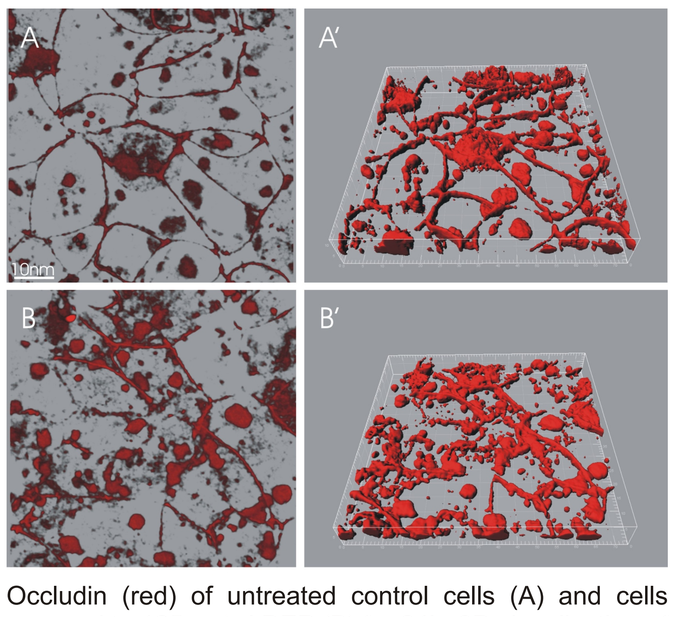
Results

Absolute occludin mRNA expression



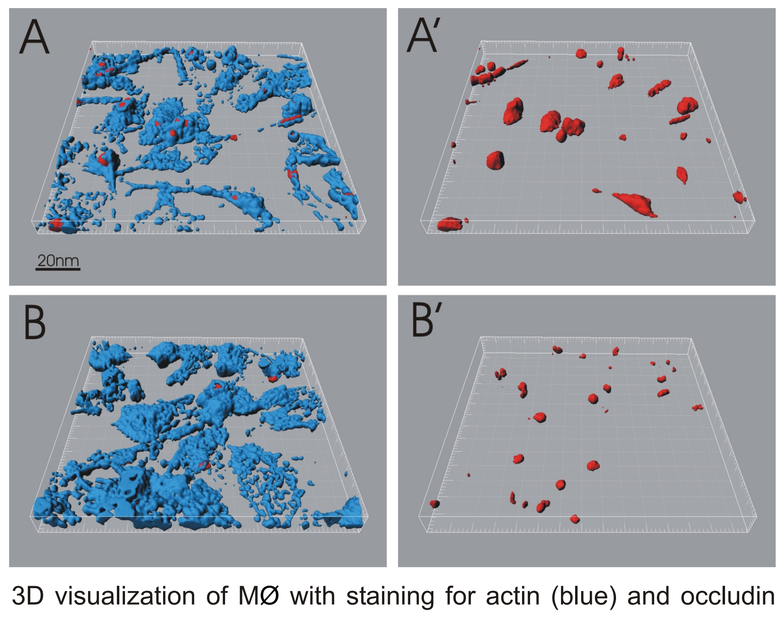
ECs have a significantly higher expression of occludin mRNA. MDM and MDDC express occludin mRNA but there is no difference between both cell types. There is no dose-response relationship but it might be the highest dose (125 g/ml) reduce the expression. (n = 3 - 7)

Occludin visualization in EC monocultures by LSM



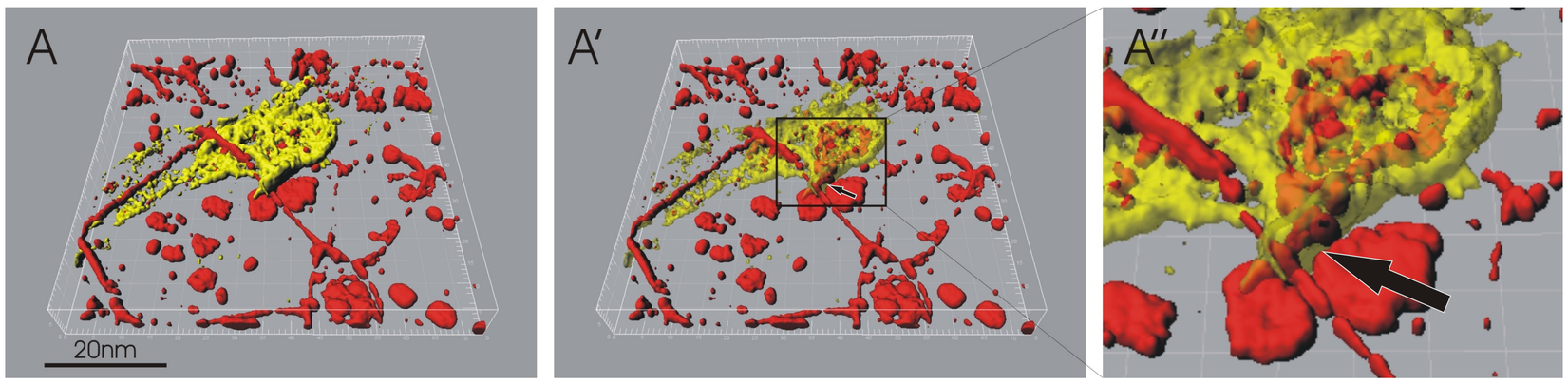
Occludin (red) of untreated control cells (A) and cells exposed to 125 g/ml DEP (B) for 24h. 3D visualization of occludin of the EC layer (A', B'). Qualitatively there is no difference in occludin amount but the structure in A seems more ordered. Actin was not affected (data not shown).

Occludin visualization in MDM monocultures by LSM

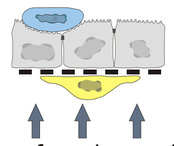


3D visualization of MØ with staining for actin (blue) and occludin (red) of untreated control cells (A) and cells exposed to 125 g/ml DEP for 24h (B). Presentation of occludin only (A', B'). Treated cells showed less occludin. Pictures were taken with same settings.

Occludin visualization of untreated co-cultures

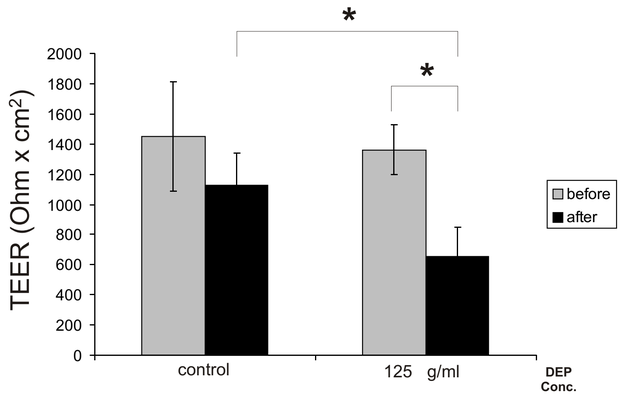


View from base side of the membrane. Staining for occludin (red) and DC (yellow). Visualization of the occludin "interplay" between occludin of ECs and them of MDDCs in A and the visualization of internal occludin in MDDCs (black arrow) in A' . Detail view in A".



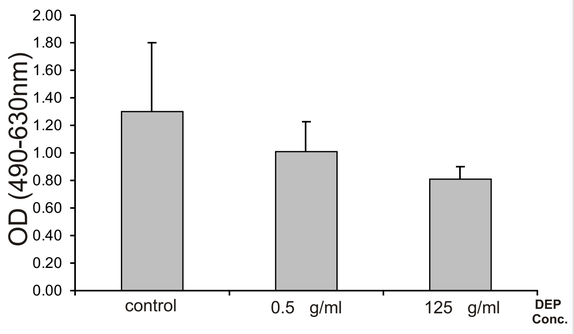
View from base side

Epithelial integrity of EC monocultures



TEER is significantly reduced when cultures are treated with DEP (24 g/ml) for 24h. (control n=3; DEP n = 4).

Cytotoxicity in DEP treated co-cultures



There is no dose effect of DEP concerning cell death (n = 3).

Conclusion

Monocultures:
We conclude that MDM and MDDC constantly express the TJ protein occludin.
LPS and DEP at concentrations from 0.5 to 50 g/ml have no effect on the mRNA expression of occludin.
DEP at high concentration (125 g/ml) modulates the expression of TJ:
- the TJ network is more irregular in treated EC cultures
- the TEER values are significantly lower in treated EC cultures
- there is less occludin protein as well as lower levels of occludin mRNA in MDM

Triple cell co-cultures:
There is no cytotoxic effect of DEP used at different concentrations.
We suggest that MDDC and EC form TJ-like complexes.

Methods

Cell Cultures
- The 16HBE14o EC line was used [4]; MDM, MDDC 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.

Cofocal Laser Scanning Microscopy (Zeiss 510 META)
- After fixation the cells were stained for F-actin and occludin and the different surface markers (CD14 for MDM and CD86 for MDDC).
- 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)
- Reverse Transcription was done using Oligo (dT)15-primers and OmniscriptRT (Qiagen)
- Real-time quantitative- PCR was done using Sybr Green Jump Start Taq Ready Mix (Sigma)
- Beta-actin was used as housekeeping gene and the data were evaluated by the relative quantification method (2-delta CT) [5]

Cytotoxicity
- Lactate dehydrogenase in the supernatants was determined (Cytoxitcity assay)

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

Literature

[1] Adapted from McWilliam AS, Holt PG (2000), Rat models of respiratory inflammation, *Methods Mol Biol*.138:303-9.
[2] Feldman GJ, Mullin JM, Ryan MP (2005), Occludin: structure, function and regulation, *Adv Drug Deliv Rev*. 57:883-917.
[3] Rothen-Rutishauser BM, Kiama SG, Gehr P (2005), A three-dimensional cellular model of the human respiratory tract to study the interaction with particles, *Am.J.Respir.Cell Mol.Biol.* 32: 281-289.
[4] Blank F, Rothen-Rutishauser BM, Schurch S, Gehr P (2006), An optimized in vitro model of the respiratory tract wall to study particle cell interactions, *J. Aerosol Med.* 19:392-405.
[5]Livak KJ and Schmittgen TD (2001), Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, *Methods*. 25:402-8.

Acknowledgments

We thank **B. Tschirren** and **S. Frank** for their excellent technical assistance.
The work was supported by...
the Swiss National Sciences Foundation (SNF)
the German Research Foundation (DFG)