# 4 Own Work

## 4.1 Importance of airway epithelial cells

Jahn, U.*, Krüll, M.*, F. N. Wuppermann, A. C. Klucken, S. Rosseau, J. Seybold, J. H. Hegemann, C. A. Jantos, and N. Suttorp. Infection and activation of airway epithelial cells by *Chlamydia pneumoniae*.
*J. Infect. Dis*. 182:1678-1687, 2000. ***5 K***

Wissel, H., C. Schulz, M. Rüdiger, M. Krüll, P. A. Stevens, and R. R. Wauer. *Chlamydia pneumoniae* affect surfactant trafficking and secretion due to changes of type II cell cytoskeleton.
*Am. J. Respir. Cell Mol. Biol*. 29:303-313, 2003. ***10 K***

Wissel, H., T. Müller, M. Rüdiger, M. Krüll, and R. R. Wauer. Contact of *Chlamydophila pneumoniae* with type II cell triggers activation of calcium-mediated NF-kappaB pathway.
*Biochim Biophys Acta.* 1743:37-48, 2005. ***14 K***

Krüll, M., P. Bockstaller, F. N. Wuppermann, A. C. Klucken, J. Mühling, B. Schmeck, J. Seybold, C. Walter, M. Maass, S. Rosseau, J. H. Hegemann, N. Suttorp, and S. Hippenstiel. Mechanisms of *Chlamydophila pneumoniae* mediated GM-CSF release in human bronchial epithelial cells.
*Am. J. Resp. Cell Mol. Biol.* 34:375-382, 2006. ***17 K***

Development of new therapeutic anti-inflammatory strategies requires a substantiated information about pathogen-host interaction. During respiratory infection, airway epithelial cells are the first line of defense getting in contact with *Chlamydophila pneumoniae*. Until now, however, only little is known about mechanisms of bronchial or alveolar epithelial cell infection and activation. Objective was to examine if *C. pneumoniae* is able to infect and subsequently activate different epithelial cells from the respiratory tract.
Infection of freshly isolated human (tracheal) airway epithelial cells, small airway epithelial cell (SAEC), freshly isolated (rat) type II pneumocytes and the epithelial cell lines BEAS-2 (bronchial epithelial cells) and A549 (alveolar epithelial cells) could be

demonstrated. In these cells, *C. pneumoniae* strongly activated expression of proinflammatory mediators (IL-8, GM-CSF, ICAM-1) in a p38 MAPK- (but not ERK1/2- or SAPK/JNK-) and NF-κB-dependent manner. Subsequently increased interaction of PMN and infected epithelium was noted. Interestingly Chlamydia-induced p38 MAPK activity seemed to be critical for NF-κB-stimulation. In addition, we identified chlamydial outer membrane protein-A (Omp-A) and heat shock protein 60 (GroEL-1) as a key virulence factor and show that recombinant purified chlamydial GroEL-1 protein sufficiently activated a NF-κB-dependent GM-CSF expression in epithelial cells.

In addition, by demonstrating a *C. pneumoniae*-mediated expression of COX2 in airway epithelial with subsequent release of $PGE_2$ we suggest, that epithelial cells may play a dual role during infection and inflammation, dampening a proinflammatory situation.

Confocal laser scanning microscopic (CLSM) examinations of alveolar epithelial cells (typ II pneumocytes, A549) demonstrated, that *C. pneumoniae* colocalized with surfactant protein (SP)-A–mediated endocytosed lipid and early endosomes (EEA1- and Rab5-positive). Moreover, SP-A-mediated lipid uptake and accumulation was significantly increased. SP-uptake and accumulation could be attributed to an inhibition of intracellular surfactant transport via a *C. pneumoniae*-mediated alteration of epithelial β-tubulin (accumulation) and F-actin (secretion). Moreover, CLSM-data suggested, that these mechanism of cytoskeleton reorganization as well as (subsequent) NF-κB activation in type II cells is $[Ca^{2+}]i$ dependent.

Our observations add important new properties to this bacterium, i.e., its capacity to induce a cascade of events leading to a profound (pro- and anti-) inflammatory activation of airway epithelial cells. *C. pneumoniae*-induced epithelial cytokine liberation with subsequently enhanced interaction of leukocytes and epithelial cells may contribute significantly to inflammatory airway diseases like chronic obstructive pulmonary disease (COPD) or bronchial asthma.

## 4.2 Importance of endothelial cells

### 4.2.1 Endothelial cells as targets of pathogens

Krüll M., C. Dold, S. Hippenstiel, S. Rosseau, J. Lohmeyer, and N. Suttorp. *Escherichia coli* hemolysin and *Staphylococcus aureus* alpha-toxin potently induce neutrophil adhesion to cultured human endothelial cells.
*J. Immunol.* 157:4133-4140, 1996. ***1 K***

Krüll, M., R. Nöst, S. Hippenstiel, E. Domann, T. Chakraborty, and N. Suttorp. *Listeria monocytogenes* potently induces upregulation of endothelial adhesion molecules and neutrophil adhesion to cultured human endothelial cells.
*J. Immunol.* 159:1970-1976, 1997. ***2 K***

Schwarzer, N., R. Nöst, J. Seybold, S. K. Parida, O. Fuhrmann, M. Krüll, R. Schmidt, R. Newton, S. Hippenstiel, E. Domann, T. Chakraborty, and N. Suttorp. Two distinct phospholipases C of *Listeria monocytogenes* induce ceramide generation, nuclear factor-kappa activation, and E-selectin expression in human endothelial cells.
*J. Immunol.* 161:3010-3018, 1998. ***3 K***

Hippenstiel S, S. Soeth, B. Kellas, O. Fuhrmann, J. Seybold, M. Krüll, C. v. Eichel-Streiber C, M. Goebeler, S. Ludwig, and N. Suttorp Rho proteins and the p38-MAPK pathway are important mediators for LPS-induced interleukin-8 expression in human endothelial cells.
*Blood* 95:3044-3051, 2000. ***6 K***

Fuhrmann, O., M. Arvand, M. Krüll, S. Hippenstiel, J. Seybold, C. Dehio and N. Suttorp. *Bartonella henselae* outer membrane proteins (omp) induce NF-κB-dependent upregulation of adhesion molecules and subsequent neutrophil adhesion to human endothelial cells.
*Infect. Immun.* 69:5088-5097, 2001. ***7 K***

Walter, C., J. Zahlten, B. Schmeck, C. Schaudinn, S. Hippenstiel, E. Frisch, A. C. Hocke, N. Pischon, H. K. Kuramitsu, J. P. Bernimoulin, N. Suttorp, and M. Krüll. *Porphyromonas gingivalis* strain-dependent activation of human endothelial cells.
*Infect. Immun*. 72:5910-5918, 2004. ***11 K***

The endothelium lines the inner surface of the vessel wall and is exposed to pathogens, pathogen-derived products as well as to agents of the activated host defense in almost all (local or systemic) inflammatory responses.

In previous studies, we could identify several pathogens (*Listeria monocytogenes*, *Bartonella henselae*, *Porphyromonas gingivalis*) or bacterial products/virulence factors (*E. coli* hemolysin, *Staph. aureus* $\alpha$-toxin, *L. monocytogenes* hemolysin and phospholipases, *B. henselae* outer membrane proteins, *Salmonella abortus equii* lipopolysaccharide) to activate key intracellular signal transduction pathways (increase of $[Ca^{2+}]i$, activation of small GTP-binding proteins of the Rho-family, increase of sphingomyelin/ceramides, phosphorylation of p38, ERK1/2 and SAPK/JNK, activation and transnuclear shift of NF-$\kappa$B) in freshly isolated human endothelial cells. Expression of a sustained proinflammatory phenotype in endothelial cells was noted (release of IL-6, IL-8, RANTES, PAF, sequential expression of endothelial adhesion molecules P-selectin, E-selectin, ICAM-1 and VCAM-1). A subsequently enhanced interaction (rolling, adhesion, transmigration) of leukocytes (PMN, monocytes) and activated endothelial cells could be demonstrated.

Overall, until now many pathogens or pathogen derived virulence factors have been identified to activate preformed, stereotypic signal transduction pathways and proinflammatory mechanisms in endothelial cells. Future research is now necessary to identify pathogen-specific characteristics of target cell infection and activation and to possibly develop new (non-antibiotic) therapeutic strategies.

### 4.2.2 Endothelial cell infection and activation by *C. pneumoniae*

Krüll, M., A. C. Klucken, F. N. Wuppermann, O. Fuhrmann, C. Magerl, J. Seybold, S. Hippenstiel, J. H. Hegemann, C. A. Jantos, and N. Suttorp. Signal transduction pathways activated in endothelial cells following infection with chlamydia pneumoniae.
*J. Immunol.* 162:4834-4841, 1999. ***4 K***

Krüll, M., J. Kramp, T. Petrov, A. C. Klucken, A. C. Hocke, C. Walter, B. Schmeck B, J. Seybold, M. Maass, S. Ludwig, J. G. Kuipers, N. Suttorp, and S. Hippenstiel. Differences in cell activation by *Chlamydophila pneumoniae* and *Chlamydia trachomatis* infection in human endothelial cells.
*Infect. Immun*. 72:6615-6621, 2004. ***12 K***

Opitz, B., S. Förster, A. C. Hocke, M. Maass, B. Schmeck, S. Hippenstiel, N. Suttorp, and M. Krüll. Nod1-mediated endothelial cell activation by *Chlamydophila pneumoniae*.
*Circ. Res*. 96:319-326, 2005. ***15 K***

Krüll, M., M. Mass, N. Suttorp, and J. Rupp. *Chlamydophila pneumoniae* – mechanisms of target cell activation.
*Thromb. Haemost.* 94:319-326, 2005. ***16 K***

Krüll, M., F. N. Wuppermann, C. Scheiber, J. Kramp, J. Seybold, J. Mühling, M. Maass, J. H. Hegemann, N. Suttorp, and S. Hippenstiel. *Chlamydophila pneumoniae* heat shock protein-60 mediated target cell activation.
*Infect. Immun.* In reply. 2006. ***18 K***

Chronic *C. pneumoniae* infection has been suggested as a trigger/promoter of inflammation that may result in vascular lesions. The theory is supported by a serological association between *C. pneumoniae* infection and vascular diseases (CHD, AOD, carotid artery stenosis and stroke), the demonstration of *C. pneumoniae* in atherosclerotic plaques by electronmicroscopy, IHC, PCR, and isolation of viable chlamydia (indicating a productive chlamydial infection), and different animal models, demonstrating that intranasal infection of mice and rabbit with *C. pneumoniae* leads to pneumonia, perimyocarditis, septic circulatory dysregulation and - more delayed -

systemic spread of chlamydia into spleen, lymphnodes, peritoneum and atherosclerotic plaques of arterial blood vessels. Atherosclerotic lesions develop upon chronic inflammatory reactions of the endothelial cells and the vascular intima (“response to injury”-theory ). The role of *C. pneumoniae* in atheroma formation has not been studied in detail.

We were able to identify endothelial cells as primary target cells for infection with *Chlamydophila pneumoniae*. In these cells, *C. pneumoniae* induced a prolonged pro-inflammatory phenotype with an enhanced release of IL-8, MCP-1, RANTES and IL-6 as well as an upregulation of endothelial adhesion molecules E-selectin, ICAM-1, and VCAM-1 followed by subsequently increased interaction (rolling, adhesion, transmigration) of PMN and monocytes with infected EC. In this context, importance of key intracellular signal transduction pathways (increase of $[Ca^{2+}]i$, activation of small GTP-binding proteins of the Rho-family, phosphorylation/activation of p38/ERK1-2/SAPK, PKC, activation and transnuclear shift of NF-κB) could be demonstrated. Using recombinant chlamydial heat shock protein 60 (GroEL-1), it turned out, that this protein is key virulence factor for *C. pneumoniae*-mediated endothelial cells activation. In a recent study, intracellular endothelial Nod1-protein could be identified as pivotal receptor for viable intracellular *Chlamydophila pneumoniae*, triggering activation of inflammatory processes. Moreover, we showed, that effects on endothelial cell activation involved in the development of cardio-vascular diseases were specific for *C. pneumoniae* since infection of these cells with *Chlamydia trachomatis* did not induce phosphorylation and activation of MAPK or expression of proinflammatory and proartherogenic marker (no release of endothelial IL-8, no upregulation of ICAM-1).

Ongoing studies using new techniques of biochemical and genetical analysis (genomics, proteomics, epigenetics), will now improve our understanding about (specificity of) *C. pneumoniae*-mediated pathomechanisms of infection and inflammation.

### 4.2.3 Signal transduction pathways activated in target cells

Krüll, M., M. Mass, N. Suttorp, and J. Rupp. *Chlamydophila pneumoniae* – mechanisms of target cell activation.

*Thromb. Haemost.* 94:319-326, 2005. ***16 K***

*Chlamydophila pneumoniae* is able to activate a multitude of signal transduction pathways in target cells. The following review will focus on the importance of putative host cell receptors for *C. pneumoniae* and subsequently activated signal transduction pathways.