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**Institutet för miljömedicin
Enheten för lung- och allergiforskning**

Toll-like Receptors in Airway Inflammation *in vitro* and *in vivo*

AKADEMISK AVHANDLING

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ABSTRACT

During evolution a variety of solutions has been developed by different living organisms in order to protect itself from invading potential pathogens and particles. Protection of the host is essential for its survival and involves efficient recognition and elimination of potential pathogens. The recognition is often mediated by pathogen recognition receptors (PRRs). Activation of PRRs is mainly driven by exogenous pathogen associated molecular patterns (PAMPs) or endogenous danger associated molecular patterns (DAMPs) and result in secretion of multiple pro-inflammatory cytokines. Toll-like receptors (TLRs) are members of the PRR family and the importance of these receptors during pro-inflammatory conditions is addressed in the present thesis, both in vitro and in vivo.

In Paper I we showed that farmers and smokers, two groups that are continuously exposed to organic material through daily work at the farm or through tobacco smoke, have an ongoing inflammation in the respiratory tract. It was shown that chronically exposed subjects develop an adaptation to the effects of acute exposure to inhaled organic material. Further, it was demonstrated that exposure in the swine barn was a much stronger pro-inflammatory stimulus than inhaled pure lipopolysaccharide (LPS), in vivo.

In Paper II, the gene expression of TLR2 on primary bronchial epithelial cells was demonstrated. This expression was synergistically enhanced by co-stimulation with pro-inflammatory stimuli and glucocorticosteroids. Dust obtained from the swine barn was a more potent pro-inflammatory stimulus than pure LPS or pure peptidoglycan (PGN), in vitro, as already shown in vivo. The secreted pro-inflammatory cytokines from the epithelial cells were diminished by blocking of the TLR2 and TLR4 with monoclonal antibodies, indicating that the pro-inflammatory stimulation was at least partly dependent on TLR2 and TLR4.

In paper III we found that TLR2 on blood neutrophils was down-regulated by pro-inflammatory stimuli, whereas the expression TLR4 and CD14 were unaffected by the pro-inflammatory stimulation, in vitro. The expression of TLR4 and CD14 were increased by the presence of epithelial cells, irrespective of stimulation. Moreover, we showed synergistically enhanced secretion of CXCL8 (IL-8) and sCD14 during co-culture compared to single culture condition and a strong positive correlation between CXCL8 and sCD14 in LPS-stimulated co-cultured cells. These findings strongly suggest an active bidirectional cross-talk between alveolar epithelial cells and neutrophils.

In paper IV we confirmed what we already had shown in vitro, that TLR2 was down regulated by pro-inflammatory conditions on neutrophils, this time in vivo. We also showed the presence of soluble TLR2 (sTLR2) in BAL and sputum and that this expression was altered in COPD compared to healthy subjects. Moreover, CD14 expression on sputum neutrophils was enhanced compared with blood neutrophils and that the gene expression of CD14 on alveolar macrophages in BAL-fluid was increased in smokers compared with non-smokers. These findings indicate that PRRs expression is altered by smoking per se, but also that the disease, COPD, contributes. This is likely of importance in COPD patho-physiology, in particular for exacerbations, which often are caused by microorganisms.

Overall, these studies have shown the involvement of PRRs in several immunological active cell types during pro-inflammatory conditions. A better understanding of the mechanisms behind PRRs regulation and outcome would potentially benefit drug development and in the end many patients with inflammatory diseases.