

Interleukin-17 in Endotoxin-Induced Airway Inflammation

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Avhandlingen baseras på delarbeten:

- I O. Prause, M. Laan, J. Lötvall, A. Lindén**
“Pharmacological modulation of interleukin-17-induced GCP-2-, GRO-alpha- and interleukin-8 release in human bronchial epithelial cells”
European Journal of Pharmacology 462 (2003) 193-198
- II M. Miyamoto, O. Prause, M. Sjöstrand, M. Laan, J. Lötvall, A. Lindén**
“Endogenous interleukin-17 as a mediator of neutrophil recruitment caused by endotoxin exposure in mouse airways”
Journal of Immunology 170 (2003), 4665-4672
- III O. Prause, S. Bozinovski, G.P. Anderson, A. Lindén**
“Increased matrix metalloproteinase-9 concentration and activity after stimulation with interleukin-17 in mouse airways”, Submitted
- IV O. Prause, S. Ivanov, M. Sjöstrand, A. Lindén**
“Local IL-17 release after exposure to endotoxin in mouse airways in vivo: Sensitivity to pharmacological modulation”, As manuscript

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Chronic obstructive pulmonary disease (COPD) and acute, severe asthma are associated with a local accumulation of neutrophils. The neutrophils are believed to contribute to the pathological features of these diseases by releasing proteases and reactive oxygen free radicals that cause mucus secretion, airway remodelling and lung tissue destruction.

Interleukin (IL)-17 is a T lymphocyte derived cytokine that may play a role in the recruitment and activation of neutrophils in the airways. Recent studies have shown that IL-17 is elevated in asthma and in airway inflammation induced by exposure to organic dust.

The general aim of the current studies was to determine the role of IL-17 in endotoxin-induced neutrophil recruitment and activation. The role of IL-17 was evaluated using *in vivo* a mouse endotoxin model and *in vitro* cultures of human bronchial epithelial cells and mouse neutrophils, airway macrophages and T lymphocytes.

In study one, IL-17 induced the release of several neutrophil-recruiting CXC-chemokines such as IL-8, growth-related oncogene (GRO)-alpha and granulocyte chemoattractant protein (GCP)-2 from human bronchial epithelial cells concentration dependently. The release of IL-8 was mediated by the p38 and the ERK kinase pathway, the release of GRO-alpha and GCP-2 was mediated by the p38 kinase pathway alone. Glucocorticoid receptor stimulation inhibited the IL-17 induced release of IL-8, GRO-alpha and GCP-2. Beta-2-adrenoceptor stimulation and calcineurin phosphatase inhibition did not cause an inhibitory effect on CXC chemokine release. These results indicate that stimulation with IL-17 induces the release of several neutrophil recruiting cytokines that hypothetically need to be targeted to modify neutrophilia. Glucocorticoid receptor stimulation constitutes one way to inhibit the release of several CXC-chemokines.

In study two, intranasal administration of endotoxin increased the concentration of IL-17 locally. Inhibition of IL-17 in endotoxin-induced airway inflammation attenuated neutrophil recruitment in association with decreased concentrations of IL-6 and macrophage inflammatory protein (MIP)-2 in BAL fluid. *In vitro*, isolated CD3+ lymphocytes released IL-17 after stimulation with endotoxin, when cocultured with mouse airway macrophages. These findings suggest that IL-17 mediates the endotoxin-induced neutrophil recruitment and that T-cells constitute a source for IL-17.

In study three, local stimulation with IL-17 increased the concentration of proMMP-9 in mouse airways in association with a pronounced local accumulation of neutrophils that stain positive for MMP-9. Stimulation with IL-17 also increased the concentration of free, soluble MMP-9, which was proteolytically active as determined by a gelatinase substrate assay. *In vitro*, stimulation of mouse neutrophils with IL-17 did not increase the concentration of proMMP-9 in the conditioned medium. These findings indicate a link between the T-cell cytokine IL-17 and increased proteolytic load in the airways that may be of relevance for diseases like severe asthma and COPD.

In study four, stimulation of the glucocorticoid receptor, the beta-2-adrenoceptor and inhibition of the phosphatase calcineurin, respectively, inhibited endotoxin-induced neutrophil recruitment in association with decreased concentrations of IL-17. *In vitro*, the glucocorticoid hydrocortisone and cyclosporin A caused inhibition of endotoxin-induced IL-17 release from a coculture of T lymphocytes and airway macrophages. This was not the case for salbutamol. Taken together, these findings suggest that the endotoxin-induced IL-17 release is sensitive to a glucocorticoid, a beta-agonist and calcineurin phosphatase inhibition *in vivo*.

In conclusion, the cytokine IL-17 plays a central role in endotoxin-induced neutrophil recruitment into mouse airways. Therefore may IL-17 constitute a potential target in neutrophil-associated airway diseases like COPD and chronic, severe asthma. Further studies have to be done to determine the role of IL-17 in neutrophil recruitment in human airways

Key words: IL-17, airway inflammation, endotoxin, T lymphocytes, neutrophils, secondary mediators, MMP-9.

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