6. Summary

In vitro- and ex vivo- investigations of cytokine expression in the equine tracheal epithelium

These studies were undertaken to explore the role of viral infection of the equine tracheal epithelium and virally induced cytokine expression. The main target was a cell culture obtained from equine tracheal epithelial cells (ET cells). The ET cell line was established in the institute for veterinary pathology. It is the second permanent cell line of equines achieved worldwide.

ET cells were infected with equine herpesvirus type 1, 2 and 4 (EHV-1, -2, -4); equine rhinitis virus serotype A (strains Perv and 350/72) and serotype B (strain P 1436/73); vesicular stomatitis virus and with equine arteritis virus. After primary infection, the cells were passaged two times and growth kinetics were evaluated for all viruses. All viruses (except EHV-4) led to productive infection of ET cells. These findings confirm the ability of equine rhinitisviruses to infect the lower airways which should be investigated in following studies. Additionally, the results provide further evidence of the infectious potential of EHV-2 as a respiratory pathogen for the lower airways of the horse. Interestingly EHV-4 was not able to set up a productive infection cycle in ET cells. We presumed a restricted persistent infection which should be subject to further investigation.

Virally induced expression of cytokines was determined by using the reverse transcription-polymerse chain reaction (RT-PCR). Total cellular RNA was extracted from ET cells, reverse transcripted in cDNA and amplified with cytokine specific primers for equine interleukin (IL) 1α, 1β, 2, 4, 5, 6, 8, 10, 11, 13, interferon gamma (IFNγ), tumour necrosis factor alpha (TNFα) and granulocyte-macrophage colony-stimulating factor (GM-CSF). ET cells expressed mRNA for IL 1α, 1β, 6, 8, 10 and 11. All cytokines could already be measured before infection, but viral infection led to changes in cytokine expression.

An important result in this context is, that both equine rhinitis A viruses increased expression for IL 11. In following studies the use of RT-PCR should be combined with quantitative methods.

To evaluate cytokine expression in the tracheal epithelium of the horse, tracheal tissue of 15 slaughtered horses without morphological changes in trachea and lungs were sampled. Cells were obtained by gentle scraping off the tracheal epithelium layer with a scalpel. mRNA for equine IL 8 (100% of the horses), IL 2 (67%), IL 1β, 6, 10 (60%), IFNγ (53%), IL 1α (20%)

and for IL 11, TNFα and GM-CSF (13%) could be detected. mRNA for IL 4, 5 and 13, which are characteristic for an immunreaction of the th2 phenotype was not found in any horse. In contrast, expression of mRNA for IL 2 and IFNγ was present in most of the horses. This and the missing IL 4, 5 and 13 detection indicates a th1 phenotype in the tracheal epithelium of horses without morphological changes.
Cells of four horses were infected immediately after scraping with EHV-1, -2 and -4 and with equine rhinitis viruses. Interestingly, culturing of cells alone led to changes in cytokine expression. Viral influence of cytokine expression could not be evaluated because of the lack of quantitative measurement.

This work shows that the equine tracheal epithelium apparently plays a central role in immunological reactions in the pathogenesis of respiratory tract diseases. Further studies should confirm and quantify these results.