7 Summary

The equine herpesvirus type 2 (EHV-2) interleukin-10 (IL-10) homologue: Activity during acute and latent infection with special consideration on gene expression *in vivo* and *in vitro*

The aim of this work was to analyse the EHV-2 IL-10 homologue gene with special consideration on its gene expression *in vitro* and *in vivo* as well as its primary structure. Thereby it should be clarified if the EHV-2 IL-10 is related to a certain transcript class, if it is connected to a defined stage of infection and if the primary structure of EHV-2 IL-10 allows conclusions to its function.

The expression kinetics of the EHV-2 IL-10 gene were investigated by an EHV-2 IL-10 specific seminested RT-PCR following an *in vitro* infection of a permanent equine dermal cell line (ED-cells) with the EHV-2 reference strain LK-4. EHV-2 IL-10 specific transcripts were already detected 6 hours post infection (p. i.), even before the transcripts of the immediate early EHV-2 ORF-50 and early late EHV-2 Glykoprotein B appeared. Furthermore, the implementation of protein synthesis inhibitors as well as inhibitors of viral DNA replication showed that the expression of EHV-2 IL-10 occurred independently from the protein synthesis and also from viral DNA replication. Therefore the *in vitro* expression of the EHV-2 IL-10 was consistent with that of an immediate early herpes virus gene.

For the analysis of the EHV-2 IL-10 expression *in vivo*, blood samples of three naturally with EHV-2 infected horses were examined monthly over a period of one year. Parallel the EHV-2 infection stage of the horses was determined. The results of serological analysis implied hints of an existing acute EHV-2 infection, but there was no evidence of productive virus replication in the peripheral blood mononuclear cells (PBMC) of all three horses either by RT-PCR for EHV-2 ORF-50 and gB expression as markers for virus replication or by plaque assay for the detection of infectious virus in destroyed cells. From the PBMC of two horses latent EHV-2 was isolated by cocultivation, whereas the implementation of 12-O-tetradecanoylphorbol-13-acetate (TPA) had a positive effect on the reactivation of the latent virus. With the EHV-2 PCR positive PBMC of the third horse the cocultivation was always negative. Additionally Gardella gel analysis indicated that the DNA of latently infected equine PBMC contained integrated EHV-2 DNA.

In the latently infected PBMC of two horses EHV-2 IL-10 specific transcripts were detected, whereas no EHV-2 IL-10 transcripts could be found in the PBMC of the third horse. The expression in latently infected PBMC suggests that the EHV-2 IL-10 takes part in the latent EHV-2 infection. However the question about the possible implementation of EHV-2 IL-10 transcripts as a diagnostic marker for the latent EHV-2 infection cannot be answered

conclusively. Possible EHV-2 IL-10 functions in latently infected equine PBMC and ways of further examinations of these results will be discussed.
Comparative studies of the EHV-2 IL-10 primary structure of 9 EHV-2 reference strains and field isolates revealed a high level of amino acid conservation of 96 % to 100 %. Moreover the EHV-2 IL-10 matches the equine IL-10 protein in 85 % of the amino acids. In addition phylogenetic analysis of EHV-2 IL-10 and equine IL-10 were conducted on basis of a developed phylogenetic tree. As a result both IL-10 proteins showed a high degree of homology, suggesting that the EHV-2 IL-10 was captured from the horse during evolution. An amino acid alignment with EBV, parapoxvirus orf, human and ovine IL-10 was carried out to identify candidate functional domains of EHV-2 IL-10. As a result regions were detected, which posses immune stimulating functions in the cellular IL-10 protein, that had not been preserved in the EHV-2 IL-10 as well as the EBV IL-10. Possibly EHV-2 IL-10, like EBV IL-10, preserved only the immunosuppressive qualities of the host IL-10, whereby a therapeutical implementation of the EHV-2 IL-10 would be conceivable with immunopathological diseases of the horse.

In conclusion, the results of the current work show that the established EHV-2 IL-10 specific snRT-PCR is a sensitive and specific tool for the verification of EHV-2 IL-10 expression. This method presents an opportunity for further analysis of the role of the EHV-2 IL-10 *in vivo*. Indications for immunomodulatory effects and probably functional differences to the equine counterpart as observed by computer based sequence analysis, have to be verified by functional studies in adequate model systems.