VI. SUMMARY

Cloning of IFNγ and IL-4 in zoo animals and an approach to determine expression levels of these cytokines by real-time PCR

The detection of cytokines provides valuable information about the nature of the host response to infections. At the present study, a sequence-based species-specific real-time RT-PCR was established to measure equine mRNA expression levels of IFNγ and IL-4 as key cytokines, involved in Th1/Th2 immune regulations. To have the opportunity to expand this test to wildlife species, cloning of IFNγ and IL-4 cDNA from PBMC of different zoo species such as Somali wild ass (*Equus africanus somalicus*), Grevy's zebra (*Equus grevyi*), Hartmann's mountain zebra (*Equus zebra hartmannae*), Indian one-horned rhinoceros (*Rhinoceros unicornis*), Asian elephant (*Elephas maximus*), European bison (*Bison bonasus*), African buffalo (*Syncerus caffer*) and Nubian giraffe (*Giraffa camelopardalis)* was performed. While IFNγ sequences revealed a significant conservation of 72-79% between humans and analysed species, IL-4 showed a higher degree of divergence (only 62-64% homology). For horse as a model animal, species-specific primers and probes were designed to establish a real-time RT-PCR. The limits of detection were found to be as low as one molecule of DNA using IFNγ or IL-4 plasmids. Sample-to-sample variation in the efficiency of the RT, as well as in the quantity and quality of the starting RNA, was normalized to the endogenous housekeeping gene 18S rRNA. The assay was applied to monitor the kinetics of IFNγ and IL-4 mRNA expression *in vitro* after mitogenic or anti-equine CD3 mAb stimulation, supported by anti-CD28 co-stimulation. Thereafter, recall antigens typical for horse vaccination, *Equine herpesvirus-1 (EHV-1)* and tetanus toxoid (TT), were applied *in vitro*. Cytokine mRNA kinetics of the mitogen-stimulated PBMC demonstrated a rapid and stable increase in IFNγ mRNA expression, and a rapid but transient increase in IL-4 mRNA expression. *EHV-1* and TT stimulation resulted in a peak mRNA expression for both cytokines within 12 hours of activation. The high sensitivity and specificity of such real-time quantitative PCR assays could make them a valuable tool to study immunologic responses and cytokine profiles of zoo and wildlife species in the future allowing the analysis of even large numbers of samples over a broad detection range.