# 7. Summary


## Cloning and expression of eq.IFNγ, eq.GM-CSF and eq.IL-4 and their influence on monocytic horse cells

In this thesis, dendritic cells (DC) should be isolated from the skin of horses. Additionally monocytes should be differentiated to DC and characterised. For this purpose, it was necessary to produce the equine (eq.) cytokines, granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin 4 (IL-4) and interferon γ (IFNγ).

With the help of consensus primers from other species, eq.GM-CSF was amplified and produced. After the RACE-procedure, a point mutation (deletion) along a section of the 3´primer, which resultated in a protein sequence 8 amino acids longer than other known species, was identified. The published sequence information of eq.IL-4 was revealed to be incorrect because it contained deletions. Only the sequence information for eq.IFNγ -except for one point mutation- could be confirmed. To produce active recombinant proteins, both prokaryotic and eukaryotic expression systems were used. In the bacterial system an over expression of all cytokines was possible but, due to the system chosen, had no biological activity. The expressed cytokines from mammalia cells were bioactive.

Langerhans cells (LC) isolated from the epidermis were examined morphologically, however the cell yield was very low. The isolated peripheral blood monocytes (PBM) from horses were differentiated with the recombinant cytokines. These monocytic cells were characterised morphologically as well as functionally. Eq. IFNγ stimulated monocytes showed characteristics of macrophages (large adherent cells with highly regulated MHC II expression). Using light- and electron-microscopic thechniques as well as analysis of the surface proteins by flow cytometry, there was found similarity between the eq.GM-CSF and the eq.IL-4 stimulated monocytes with human MoDC (veiled cells with pseudopodia and with highly regulated MHC II/CD86 expression). Tests of the mixed leukocyte reaction showed that the MoDC possessed stimulation capacity for T cells.