

8 Summary

Expression of canine IL-4 in E.coli and mammalian cells (CHO-cells/canine chondrocytes) and development of a direct- Sandwich-ELISA for the measurement of canine Interleukin-4

The aim of this study was to find fitting target cells and transfection mechanisms and to develop a suitable method for the measurement of the canine IL-4, creating a basis for the future use of gene therapy with Interleukin-4 in the treatment of rheumatoid arthritis of dogs.

The canine IL-4 was cloned in our institute and encloned in a transfection vector for mammalian cells (pcDNA3.1). Numerous studies proved cartilage cells to be particularly suitable as target cells for a transfection and for a following transplantation into the infected joint. As it was impossible to commercially acquire a canine cartilage cell line, in the context of this study primary canine cartilage cells were used. For the transfection CHO cells were used as reference cells. The transfection was carried out with cationic lipids (LipofectAMINE-PLUS™). They allowed a high degree of transfection efficiency; at the same time the potential danger, which can originate in viral vectors, could be eliminated.

For the qualitative registration of the transfection efficiency the gene for canine IL-4 was also encloned in a GFP plasmide with the aim to achieve a fluorescence-microscopic representation of the transfection. The transfection efficiency amounted to about 35 %.

In addition the mRNA of the transfected CHO cells and cartilage cells was gained with the help of the TriStar reagent and proved with RT-PCR.

For the quantitative measurement of the expression of IL-4 the double sandwich ELISA was developed. To reach a higher sensitivity the enforcement system Biotin/Streptavidin was applied. The utmost sensitivity degree amounted to 0.1 ng/ml = 5 pg protein mass.

The measurements of IL-4 in the supernatant of the transfected cartilage cells showed that it was possible to gain up to 260 ng/ml expressed IL-4 from about 10^2 cartilage cells.

In a further step it will have to be shown that the high expression degree of the IL-4 of the transfected cartilage cells is sufficient to achieve acceptable therapeutic effects in the infected joint. If this can be proven, a decisive step towards the development of a gene therapy against rheumatoid arthritis of the dog will be done.

Summary

Gene therapy is considered to be a promising concept of treatment in many ranges. A high scientific interest in this method of treatment consists especially in the field of orthopaedics. The specific use of the cytokine Interleukin-4, which has an anti-inflammatory effect, for the treatment of rheumatoid arthritis of humans is regarded as a promising approach.