

## 6. Summary

The use of species-specific ELISAs and bioassays for the purpose of detecting pyrogenic contaminations

In this study the reaction of the blood monocytes of rabbits and humans towards pyrogens (fever-inducing substances) was compared. Pyrogens in intravenously applied parenterals are being recognized by blood monocytes and induce the production of cytokines which cause a fever reaction in the organism. The fever reaction of rabbits has been used since 1942 for testing drugs for pyrogenic contaminations. A new in vitro pyrogen test (IPT) which measures the cytokine production of human monocytes in vitro in an ELISA system was recently validated and introduced. ELISAs for the rabbit were developed in order to compare the reaction of both species in vitro.

The first question that was addressed was whether the cytokine production of the monocytes of the rabbit corresponded to the in vivo fever reaction. This was the case. The required amount of pyrogen in vivo corresponded exactly to the amount in vitro. Having that established, the in vitro pyrogen test using human whole blood was compared to the reaction of rabbit whole blood. The human and the rabbit whole blood test were performed in parallel, whenever possible. A comparison of these two assays revealed a very high similarity in the reactivity between both species, but the rabbit appeared less sensitive towards 3 stimuli. The humans had a more uniform response and sensitivity towards all stimuli used than had the rabbits, the different donors as well as the different endpoints, IL- $\beta$ , IL-6, TNF- $\alpha$  and IL-8. The amount of pyrogens that was necessary to evoke a cytokine production varied between picograms, nanograms and even micrograms depending on the stimulus. Since the dog as well had once been discussed as an animal for pyrogen testing, a bioassay for canine blood was established and successfully compared to the corresponding bioassay using human blood.

Testing for pyrogenic contaminations is a major issue in human medicine. The in vitro pyrogen tests reliably detect pyrogenic contaminations in parenterals. From our perspective, the already established readouts, and, if necessary, further developments of species-specific ELISAs and bioassays could prove valuable for ensuring drug safety in the veterinary field.