Development of an Enzyme-linked immunosorbent assay (ELISA) for the measurement of canine C-reactive protein

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Introduction

The C-reactive protein (CRP) takes part in the acute phase reaction and assists diagnosing inflammatory processes in human as well as in canine patients. Molecular and antigenetic differences between canine and human CRP impede the detection of canine CRP (cCRP) with available human detection systems. Therefore the development of specific canine assays, which are supposed to be useful diagnostic tools concerning inflammatory canine diseases, is required.

The aim of the project was to develop an ELISA with a newly generated rabbit anti-cCRP antibody.

Material and Methods

1. Purification of cCRP from serum samples of diseased dogs was performed by
   I. fast liquid chromatography (FPLC), using a phosphoryl-cholin column (ppc-column) (Fig. 1)
   II. anion exchange chromatography and
   III. hydrophobic interaction chromatography (HIC)

2. Anti-cCRP antibodies were raised by immunization of two rabbits and attained with a cCRP column.

3. The ELISA was created as a two site sandwich assay, binding the cCRP to one anti-cCRP antibody coated to a microtiter plate and a second biotinylated cCRP detection antibody in a buffer solution. The evaluation of the ELISA was performed with serum samples of 12 healthy and 54 dogs with different inflammatory and neoplastic processes.

4. The detection accuracy was determined by a commercial ELISA (Tridelta Development Ltd, Ireland) validated for canine samples as a reference method.

Results

The range of the standard curve for the new cCRP ELISA is between 50 and 500 ng/ml (Fig. 3). Therefore samples with a cCRP content between 5 and 50 mg/l may be detected using a 1:100 predilution. For a protein content above 50 mg/l a higher dilution of 1:200 to 1:500 is necessary.

All healthy dogs investigated had CRP values below the commonly defined cut-off of 10 mg/l. In contrast to the healthy dogs, 44 of the diseased dogs with inflammatory and neoplastic processes showed much higher cCRP concentrations up to 250 mg/l.

Discussion

The reliable detection of inflammatory processes plays an important role in diagnostic processes, not only in human, but also in veterinary medicine. Therefore consistent and species specific detection assays are necessary. This study indicates that the generated ELISA using a canine specific anti-CRP antibody has a high correlation with the Tridelta ELISA which is, at the moment, the gold standard in detecting the canine inflammatory protein CRP.