Enzyme-Linked Immunosorbent Assay for Detection of Feline Serum Amyloid A Protein by Use of Immunological Cross-Reactivity of Polyclonal Anti-Canine Serum Amyloid A Protein Antibody

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ABSTRACT. Immunological cross-reactivity between feline and canine serum amyloid A protein (SAA) was studied to establish enzyme-linked immunosorbent assay (ELISA) with heterologous antibody. Purified feline SAA formed a precipitin line in immunodouble diffusion with anti-canine SAA antibody. Immunological cross-reactivity was similarly observed in ELISA. In sandwich ELISA with anti-canine SAA antibody, a dose-response curve was obtained over the range of 2 μg/ml to 123 μg/ml of purified feline SAA. From the present findings, the sandwich ELISA is found to have high repeatability for quantitation of purified feline SAA and may be applicable to determine the serum concentration of feline SAA. — KEY WORDS: ELISA, feline, SAA.


The concentrations of several serum proteins are known to increase after microbial infection, inflammatory stimulus, and neoplastic and traumatic disorders [2, 10]. Hence, these serum proteins are defined as acute phase proteins and are supposed to play an important role(s) in the nonspecific defense mechanism of animals [12]. C-reactive protein (CRP), serum amyloid P component (SAP), serum amyloid A protein (SAA), haptoglobin (Hp), and α1-acid glycoprotein (α1-AG) are known as acute phase proteins. Although SAA is one of the major acute phase proteins in human and animals [3, 6, 8, 11, 14], it is an apolipoprotein in high-density lipoprotein fraction of serum which is a poor immunogen of low molecular weight [1, 4]. Antibodies with high-titers are required to measure the SAA concentration in animal sera by single radial immunodiffusion (SRID). Thus, attempts were made to establish sandwich enzyme-linked immunosorbent assay (ELISA) which is more sensitive and reproducible than SRID to study whether SAA is one of the feline acute phase reactants or not.

Both canine and feline sera were collected from clinically normal mongrel dogs and cats 24 hr after intramuscular injection with turpentine oil (0.5 ml/kg of body weight) as described previously [9].

According to the procedure for isolation of human SAA [4], both canine SAA and feline SAA were isolated from respective pooled sera by ultracentrifugation followed by gel filtration. The molecular weights of purified canine and feline SAA were estimated to be 15,500 and 16,000, respectively by sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis [5]. For preparing an immunogen, purified canine SAA was mixed with bovine serum albumin (BSA, Sigma Chemical Co., St. Louis, MO, U.S.A.) at a molar ratio of 10:1 and its pH was adjusted to 4.5-5.0 with 0.1N HCl. 1-ethyl-3-(3-dimethyl-amino-propyl) carbodiimide hydrochloride (Sigma Chemical Co.) was added to the mixture to a final concentration of 10 mg/ml. The mixture was allowed to stand at room temperature for 2 hr.

For production of rabbit anti-canine SAA antibody, 1 ml of the BSA-coupled canine SAA (2 mg/ml) was emulsified in 1 ml of Freund’s complete adjuvant and injected subcutaneously into white rabbits. This procedure was repeated twice for every 2 weeks. Two weeks later, 250 μl of the immunogen without adjuvant was injected. Specific antibody against purified canine SAA was isolated from rabbit immune serum by affinity chromatography on purified canine SAA-coupled Sepharose 4B. Biotinylation of affinity-purified anti-canine SAA antibody was carried out as follows: 1 ml of purified anti-canine SAA antibody (1 mg/ml) in 0.1 M NaHCO3 was incubated with 60 μl of biotinyl-1-N-hydroxysuccinimide ester (1 mg/ml, EY Laboratories, San Mateo, CA, U.S.A.) for 3 hr at room temperature. Then, the mixture was dialyzed against 0.15 M phosphate buffered saline (pH 7.2, PBS) and stored at 4°C until used.

ELISA was performed using microtiter ELISA plate (Corning Inc., New York, N.Y., U.S.A.). One hundred μl of diluted purified SAA in 0.05 M carbonate buffer (pH 9.6) was added to each well of the ELISA plate and allowed to stand at 4°C overnight. After washing the plate, 200 μl of 4-fold diluted Block Acc (Dai-Nippon Pharmaceutical Co., Osaka, Japan) was added to each well and the plate was incubated at 37°C for 1 hr. After washing with PBS containing 0.05% Tween 20 (PBST), 100 μl of horseradish peroxidase-coupled goat anti-rabbit IgG (2,000-fold diluted, EY Laboratories) was added to each well. After incubation at 37°C for 1 hr, the plate was washed similarly with PBST. One hundred μl of 0.05 M citrate-phosphate buffer (pH 4.0) containing 0.2 mM 2,2’-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma Chemical Co.) and 0.04% H2O2 was added to each well. Fifteen min later, the reaction was stopped by adding 50 μl of 1 N H2SO4. The enzyme-substrate reaction was determined with a Microplate Reader (Bio-Rad Laboratories, Richmond, CA, U.S.A.). For sandwich ELISA, on the other hand, 100 μl of purified
Table 1. Immunological cross-reactivity of feline SAA with anti-canine SAA antibody determined by ELISA

<table>
<thead>
<tr>
<th>Absorbance at 405 nm</th>
<th>canine SAA</th>
<th>feline SAA</th>
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<tbody>
<tr>
<td>10 µg/ml</td>
<td>0.681 ± 0.011</td>
<td>0.356 ± 0.024</td>
</tr>
<tr>
<td>1 µg/ml</td>
<td>0.380 ± 0.014</td>
<td>0.243 ± 0.010</td>
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Purified SAA (10 µg/ml) was coated in each well of ELISA plate. a) Concentrations of anti-canine SAA antibody used for ELISA. Absorbance at 405 nm indicates absorbance of sample at 405 nm — absorbance of blank at 405 nm. Data are expressed as mean ± SD.

Table 2. Precision assays of sandwich ELISA for determination of feline SAA concentrations

<table>
<thead>
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<th>Same-day assay (n=10)</th>
<th>Different-day assay (n=5)</th>
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<tbody>
<tr>
<td>Mean (µg/ml)</td>
<td>Mean (µg/ml)</td>
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<tr>
<td></td>
<td>SD</td>
<td>CV (%)</td>
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<td>17.7</td>
<td>3.9</td>
<td>22.3</td>
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<tr>
<td>32.5</td>
<td>6.2</td>
<td>19.1</td>
</tr>
<tr>
<td>77.6</td>
<td>13.1</td>
<td>17.0</td>
</tr>
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</table>

For determination of feline SAA in serum, attempts were made to establish sandwich ELISA using anti-canine SAA antibody as both coating and probing antibodies. With affinity-purified anti-canine SAA antibody (10 µg/ml) as the coating antibody, a dose-response curve was obtained over the range of 2 µg/ml to 123 µg/ml of purified feline SAA and over the range of 0.5 µg/ml to 200 µg/ml of purified canine SAA (Fig. 1). By the use of same ELISA with purified feline SAA as an antigen, coefficients of variance of <23% and <22% were obtained by the same-day and different-day determinations, respectively (Table 2). Thus, the sandwich ELISA is a sensitive and reproducible assay to determine feline SAA in serum.

To study immunological cross-reactivity between canine SAA and feline SAA, immunogel double diffusion was carried out by the methods reported previously [13]. To study immunological cross-reactivity between canine SAA and feline SAA, immunogel double diffusion was carried out. A precipitin line was formed between purified feline SAA and anti-canine SAA antibody (data not shown), indicating the existence of immunological cross-reactivity between canine SAA and feline SAA. To confirm these findings, ELISA was also performed using anti-canine SAA antibody. With 10 µg/ml of purified SAA as coating antigen, absorbance of canine SAA at 405 nm was found to be about 2 times higher than that of feline SAA (Table 1). Similar findings were also obtained with 1 µg/ml of purified SAA as coating antigen (Table 1). The results indicate that feline SAA can be detected by use of rabbit anti-canine SAA antibody although the heterologous antigen-antibody reaction was found to be immunologically lower than the homologous reaction.

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REFERENCES