



Antibodies reacting to carbonic anhydrase isozymes (I and II) and albumin in sera from dogs

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ABSTRACT

IgGs to carbonic anhydrase isozymes (CA-I and CA-II) and albumin were identified in dog serum. IgG titers were determined in the sera of asymptomatic dogs, and in dogs with atopic dermatitis, diarrhea and/or vomiting, diabetes and/or pancreatitis, kidney disease, hepatic disease, and thyroid gland disease, using ELISA. Low titres of IgG-reactive CA-I, CA-II, BSA, and CSA were found in the sera of healthy beagles. Compared with healthy beagles, there was a significant difference in the titers of antibodies against CA-I in asymptomatic dogs, dogs with diabetes and/or pancreatitis, or thyroid gland disease, or hepatic disease. Compared with healthy beagles, there was a significant difference in the antibody titer of anti-CA-II IgG in asymptomatic dogs and in those with hepatic disease. There was a significant difference in the antibody titer of anti-BSA IgG between healthy beagles and dogs with hepatic disease.

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Carbonic anhydrases (CA; EC 4.2.1.1) catalyze the reversible hydration of CO₂ into HCO₃⁻ and are involved in ion transport, as well as water and electrolyte balance in various tissues (Sly and Hu, 1995). CA-I and CA-II are expressed mainly in erythrocytes and are widely distributed in the liver, pancreas, kidney, stomach, duodenum, and colon.

Autoantibodies reactive to CA-I and CA-II were first found in 32% of human patients with systemic lupus erythematosus (SLE) and in 21% of human patients with Sjögren's syndrome (Inagaki et al., 1991). To the best of our knowledge, there has been no report on antibodies against CA isozymes in dogs to date. In contrast, dogs have been shown to have detectable IgG antibodies to food ingredients, such as beef, cow's milk, chicken, and turkey (Zimmer et al., 2011).

The aim of this study was to investigate the presence of anti-CA-I, anti-CA-II, and anti-albumin IgG antibodies in the serum of dogs by means of an ELISA. We also discuss whether these antibodies are autoimmunity- or food allergy-related.

Canine CA isozymes were purified in our laboratory (Nishita et al., 2000). BSA (Bio-Rad Laboratories, Hercules, CA) was used, and CSA was further purified from canine albumin (Molecular Innovations, Inc., Novi, MI).

All experiments complied with the guidelines of Ethics Committee of Azabu University, who reviewed and approved this study (Approval number 090831-2, 22/2/2010). All serum samples were obtained in accordance with the guidelines of the Laboratory Animal Care Committee

of Azabu University, Japan, and programs accredited by the Office of Laboratory Animal Welfare (OLAW) USA (#A5393-01) were used.

Twenty-five male Beagle serum samples, used for healthy controls in this study, obtained from 1-month-, 3-months-, 6-months-, 12-months-, and 24-months-old animals, were obtained from the Institute for Animal Reproduction (Tochigi, Japan). As test samples, canine serum samples (from 0.5 to 22-years-old animals; *n* = 72) were randomly sampled from small animal hospitals in the Kanagawa prefecture (Japan). The subjects were asymptomatic dogs, in which no disease had been identified clinically, as well as dogs with atopic dermatitis, diarrhea and/or vomiting, diabetes and/or pancreatitis, kidney disease, hepatic disease, or thyroid gland disease.

Antibody titers against CA-I, CA-II, CSA, and BSA in canine serum were determined using ELISAs. Each well on a micro-ELISA plate (NUNC, Rochester, NY) was coated with 500 ng of CA-I, CA-II, CSA, or BSA dissolved in 0.15 M phosphate-buffered saline (0.9% NaCl) solution (pH 7.2; PBS) at 4 °C for 16 h. The plates were then washed 3 times with 0.4 mL of PBS. After incubation at 23 °C for 15 min with SuperBlock Blocking Buffers (Thermo Fisher Scientific Inc.), each well was washed 3 times with 0.4 mL PBS containing 0.5% Tween 20 (PBS-Tween). Canine serum samples were diluted with SuperBlock Blocking Buffer, diluted 10-fold and containing 0.05% Tween 20 (serum dilution buffer), and subjected to ELISA. After incubation at 26 °C for 90 min, each well was washed with PBS-Tween 5 times, and peroxidase-labeled anti-dog IgG-heavy chain antibody (Bethyl Laboratories Inc., Montgomery, TX) was diluted 12,000 times with serum dilution buffer, which was then added to the samples. After 1 h, each well was washed with PBS-

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Tween 5 times. The peroxidase activity in the wells was measured after addition of 0.1 mL of ABTS peroxidase substrate (Kirkegaard & Perry Laboratories Inc.). After 10 min, 0.1 mL of 1% SDS was added to each well to terminate the enzyme reaction, and absorbance was measured at 405 nm. All assays were performed in duplicate, and the specific binding of serum antibodies to CA-I, CA-II, BSA, and CSA was calculated as follows: the average absorbance of the antigen-coated wells minus the average of the control wells.

The specificity of the ELISA for canine IgG against CA-I, CA-II, CSA, and BSA was established by the dilution test. To determine whether some components interfered with the assay, several volumes of 3 canine serum samples were assayed for canine CA-I, CA-II, CSA, and BSA with the total volume being made up to 0.1 mL with serum diluted buffer. All serum samples were diluted 100 times with serum dilution buffer for ELISAs.

Values are expressed as the mean $\pm$ SD. Statistical differences in the titers of CA-I, CA-II, BSA, and CSA were evaluated using a one-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test. $P < 0.01$ was selected as indicating significant differences.

The titers of IgG in the sera from beagles reacting with CA-I, CA-II, BSA, and CSA are 0.30 ± 0.15 , 0.25 ± 0.10 , 0.33 ± 0.17 , and 0.22 ± 0.07 . IgG reacting with BSA in the sera of 3-month-old beagles had 14 times higher titers than that of 1-month-old dogs ($P < 0.01$). Therefore, the titers of IgG reacting with BSA in the sera of 3-month-old beagles were not used as normal controls in this study.

The titers of anti-CA-I, -CA-II, -BSA, and -CSA IgG in the sera of asymptomatic dogs and pathological dogs are shown in Table 1. Compared with beagles, there was a significant difference in the titers of antibodies against CA-I in asymptomatic dogs, dogs with diabetes and/or pancreatitis, or thyroid gland disease, or hepatic disease. Compared with beagles, there was a significant difference in the antibody titer of anti-CA-II IgG in asymptomatic dogs and those with hepatic disease. There was a significant difference in the antibody titer of anti-BSA IgG between beagles and in hepatic disease.

In the present study, high titers of IgGs against CA-I, CA-II, CSA, and BSA were found in the sera of asymptomatic and pathological dogs. The positive rate of the anti-CA isozyme antibodies in idiopathic chronic pancreatitis ranged from 26% to 33% and from 4% to 7% in the sera of normal humans (D'Cruz et al., 1996; Kino-Ohsaki et al., 1996; Andoh et al., 2002). Hilger et al. (2001) reported that anti-BSA IgGs were detected in 80–93% of an unselected human control population, insulin-dependent diabetes mellitus, and atopic patients.

Halliwell et al. (2006) reported that IgG levels were even more discriminatory than IgE values, in a study of IgG responses to 18 food antigens in dogs with cutaneous adverse food reaction (CAFR), and atopic dermatitis and healthy control dogs, because the mean rank values for 12 of 18 antigens in dogs with CAFR exceeded those of normal dogs.

Food allergens maintain their immunogenicity in spite of different treatments: many allergens are partially resistant to the effect of heat and acid, and can resist the digestion process. Guilford (1996) described that allergenicity can be influenced by food processing; protein

denaturation can destroy old epitopes (antigenic determinants) or expose new ones, resulting in a decrease or increase in allergenicity, respectively. It is not known what protein concentration, size, or structure is required to elicit an immunological reaction in a food-allergic dog (Jenkins et al., 2007).

Because the antibody against bovine CA isozymes and BSA can cross-react with canine CA isozymes and CSA, this reactive IgG may be harmful to dogs, and may pose a risk for inducing autoimmune disease.

The pathogenic mechanism of anti-CA isozyme antibodies is not known. However, Itoh and Reichlin (1992) and Nishimori et al. (1995) reported that anti-CA-II antibodies can inhibit CA-II activity, and participation of anti-CA-II antibodies in the pathogenesis of experimental autoimmune sialoadenitis and cholangitis has been demonstrated previously. Pathological lymphocyte and plasma cell infiltration was seen in the salivary glands and kidneys of CA-II-immunized mice, but not in controls (Takemoto et al., 2007). Upon acid loading, blood and urine pH decreased in both groups of mice, but the slope of the urine pH versus blood pH was less steep in the CA-II-immunized mice, suggesting that the ability of these mice to reduce urine pH during metabolic acidosis was impaired.

It is highly possible that IgGs against BSA and CA isozymes and their cross reactivity may be involved in the development and/or maintenance of clinical signs of food hypersensitivity to BSA and CA isozymes in dogs. The source of CA isozymes in pet food was likely bovine CA isozymes. The IgGs against CA isozymes and BSA in canine sera were likely induced by exposure to bovine CA-I, CA-II, and BSA in pet foods. The high titers of IgGs against CA isozymes and BSA were tended to produce in dog with hepatic disease.

It is speculated that the immunogenicity of BSA, CA-I, and CA-II was maintained in dry pet food, which led to the induction of antibody production in the dogs.

To assess the clinical importance of CA isozymes as allergens of beef components in pet food, further studies using a larger population of dogs with food hypersensitivity against beef are required.

Conflict of interest statement

None of the authors have any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Table 1

Titers of IgG react with CA-I, CA-II, BSA and, CSA in sera from dogs.

	n	Anti-CA-I	Anti-CA-II	Anti-BSA	Anti-CSA
Asymptomatic dogs	17	$0.52 \pm 0.14^*$	$0.43 \pm 0.16^*$	0.60 ± 0.44	0.32 ± 0.13
Atopic dermatitis	15	0.41 ± 0.11	0.37 ± 0.11	0.28 ± 0.24	0.28 ± 0.09
Diarrhea and/or vomiting	8	0.44 ± 0.22	0.33 ± 0.10	0.36 ± 0.38	0.33 ± 0.12
Diabetes and/or pancreatitis	7	$0.55 \pm 0.15^*$	0.41 ± 0.17	0.43 ± 0.39	0.27 ± 0.08
kidney disease	6	0.42 ± 0.18	0.36 ± 0.20	0.28 ± 0.18	0.37 ± 0.21
Hepatic disease	9	$0.69 \pm 0.19^*$	$0.52 \pm 0.12^*$	$0.85 \pm 0.68^*$	0.31 ± 0.07
Thyroid gland disease	10	$0.58 \pm 0.21^*$	0.33 ± 0.13	0.50 ± 0.57	0.30 ± 0.12

Mean $\pm$ SD.

* $P < 0.01$.

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