Identification of Bovine Serum Albumin as an IgE-Reactive Beef Component in a Dog with Food Hypersensitivity against Beef

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ABSTRACT. IgE-reactive beef components were examined by an immunoblot analysis using a serum from a dog with food hypersensitivity against beef. The immunoblot analysis revealed a distinct band at approximately 66 kDa and a faint band at approximately 50 kDa. The immunoblot analysis for serum IgE reactivity to bovine serum albumin (BSA) also revealed a positive band at 66 kDa. Serum IgE reactivity to the 66-kDa protein of beef was diminished by pre-incubating the serum sample with BSA. Furthermore, a positive reaction to BSA was detected in intradermal testing in the dog. These results clearly indicated that BSA was an IgE-reactive beef component in the dog with food hypersensitivity against beef.

KEY WORDS: allergy, beef, IgE.

NOTE

Food hypersensitivity induces nonseasonal pruritic skin diseases and gastrointestinal signs such as diarrhea and vomiting in dogs. Food ingredients such as beef, cow’s milk, soy, corn, wheat, chicken, and chicken eggs have been reported to cause food hypersensitivity in dogs [1, 3]. In the two previous reports, 60% and 73% of dogs with food hypersensitivity were shown to develop their clinical sings after oral provocation of beef, respectively [1, 3]. Thus, beef can be considered as one of the common food ingredients associated with food hypersensitivity in dogs.

Previous studies reported positive results in antigen-specific serum IgE measurement, intradermal testing (IDT), and antigen-specific histamine release assay to food antigens in dogs with food hypersensitivity [1–3], indicating that clinical manifestations of food hypersensitivity can be mediated by type I hypersensitivity in dogs that have IgE directed to food antigens in their sera. However, the antigen recognition by IgE has not been well characterized. Identification of allergic components of food is essential for elucidating the immunological mechanism and developing diagnostic tools for canine food hypersensitivity. Therefore, in the present study, we investigated beef components recognized by serum IgE in a dog with food hypersensitivity against beef by using an immunoblot analysis.

A 10-year-old female beagle that showed pruritus and erythema associated with food intake was diagnosed with food hypersensitivity against beef. The diagnosis in the dog was based on the results of a food elimination trial using a commercially available hypoallergenic diet (Prescription Diet Canine z/d ULTRA Allergen Free, Hill’s Pet Products, Topeka, KS) and the following food challenge testing using beef conducted as described previously [1, 2]. This dog was shown to have a high level of serum IgE directed to beef [46 laboratory units (LU)] by commercially available antigen-specific dog IgE tests (Topscreen and Immunodot tests, CMG Laboratories, Fribourg, Switzerland). In the healthy control dogs, levels of serum IgE directed to beef were 0 LU.

Crude beef proteins were extracted from raw beef. In brief, ground raw beef was homogenized thoroughly in phosphate buffered saline (PBS) at 4°C, and then centrifuged at 15,000 g at 4°C for 20 min. The supernatant was collected and filtrated through a 0.2-µm filter. The protein concentration of the beef extract was measured by the Bradford method standardized with bovine serum albumin (BSA).

The crude beef extract (5 µg/lane) and BSA (1 µg/lane, Sigma-Aldrich, St. Louis, MO) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) through a 5–20% gradient polyacrylamide gel under reducing conditions. The gel was then stained with the Coomassie brilliant blue staining solution. The immunoblot analysis of IgE-reactive beef components was performed using 1% cold water fish gelatin (Sigma-Aldrich) in PBS containing 0.1% Tween 20 (PBST) as a blocking buffer and an HRP-conjugated goat anti-dog IgE antibody (Bethyl Laboratories, Montgomery, TX) as a secondary antibody according to the method in our previous report [7]. In the immunoblot inhibition analysis, a diluted serum sample (1:10) in 1% gelatin PBST was pre-incubated with BSA (10 µg/ml, 100 µg/ml and 1 mg/ml) as an inhibitor at room temperature for 1 hr with gentle agitation. The following procedures were the same as those in the immunoblot analysis.

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To analyze the IgE-reactive beef components, we carried out an immunoblot analysis using a serum from the dog with food hypersensitivity against beef. In SDS-PAGE, beef extract was shown to contain a variety of proteins at various molecular weights (Fig. 1a). Serum IgE in the dog strongly reacted with an approximately 66-kDa protein of beef and weakly reacted with an approximately 50-kDa protein of beef in the immunoblot analysis. Faint bands were also detected at approximately 37 kDa and 27 kDa in the immunoblot analysis; however, these bands were considered to be non-specific binding of the anti-dog IgE antibody to the beef extract, because these bands were also found when the membrane was incubated only with the anti-dog IgE antibody control (Fig. 1b). Sera from the two healthy control dogs did not react with any beef proteins except the non-specific 37-kDa and 27-kDa bands (Fig. 1b).

Based on the result of SDS-PAGE of BSA which is a beef component (Fig. 2a), the 66-kDa protein detected in the immunoblot analysis of IgE-reactive beef components (Fig. 1b) was suspected to be BSA. We, therefore, carried out an immunoblot analysis to examine serum IgE reactivity to purified BSA in the dog with food hypersensitivity against beef. Serum IgE in the dog strongly reacted with purified BSA, whereas sera from the healthy control dogs did not (Fig. 2b). The result suggested that the 66-kDa protein found in the immunoblot analysis of IgE-reactive beef components would be BSA.

To confirm whether the 66-kDa protein of the crude beef extract that reacted with serum IgE in the dog with food hypersensitivity against beef was BSA, we performed an immunoblot inhibition analysis using BSA as an inhibitor. Serum IgE reactivity to the 66-kDa protein of the crude beef extract was diminished by pre-incubation of the serum with BSA in a dose dependent manner (Fig. 3), indicating that IgE in a serum from the dog reacted with BSA among various beef components.

Since BSA was shown to be an IgE-reactive beef compo-
nent in the dog with food hypersensitivity against beef by the immunoblot analysis, we further examined allergenicity of BSA in the same dog by IDT according to the previously described method [5]. Fifteen minutes after the intradermal injection of the beef extract (Greer Laboratories, Lenoir, NC) and BSA (1 µg/ml), positive reactions to both antigens were detected in the dog as indicated by the wheal formations comparable to the histamine control. No reactions to these antigens were observed in the healthy control dogs.

In the present study, we found that BSA was an allergen recognized by serum IgE in a dog with food hypersensitivity against beef by the immunoblot analysis and IDT. Recently, it was reported that serum IgE reactivity directed to bovine IgG was detected in dogs with cutaneous adverse food reactions to lamb, beef, and cow’s milk [4]. In the same study, it was also suggested that 51-kDa bovine phosphoglucomutase could be an allergen of beef, although serum IgE reactivity to purified bovine phosphoglucomutase was not measured [4]. In this study, we did not carry out further characterization of the IgE-reactive 50-kDa protein of beef; however, this protein might be bovine phosphoglucomutase based on its molecular weight. These results indicate that IgE directed to BSA, bovine IgG, and possibly phosphoglucomutase are involved in the development and/or maintenance of clinical manifestation of food hypersensitivity against beef in dogs as a consequence of type I hypersensitivity. To assess the clinical importance of BSA, bovine IgG, and phosphoglucomutase as allergens of beef components, further studies using a larger population of dogs with food hypersensitivity against beef are required.

Our recent study revealed that BSA was an IgE-reactive vaccine component in dogs that developed allergic reactions after vaccination [7]. Furthermore, we also found that commercially available vaccines for dogs contained a large amount of BSA [8]. Considering these findings with the results obtained in this study, it is speculated that food hypersensitivity against beef might be clinically associated with allergic reactions after vaccination in dogs via BSA. It is possible that sensitization to beef in dogs with food hypersensitivity against beef could have been established by the injection of vaccines. As another possible association between vaccination and food hypersensitivity against beef, sensitization to vaccine components such as BSA might have been developed by beef intake before vaccination, since some dogs were shown to develop allergic reactions after the first vaccination and have high levels of serum IgE directed to components in the vaccines for dogs [8]. Similar clinical association between food hypersensitivity and allergic reactions following vaccination was also reported in humans via gelatin that was included in vaccines as a stabilizer and identified as an IgE-reactive vaccine component [11]. Allergic reactions after vaccination as well as food hypersensitivity are considered important practical issues in dogs [6, 9, 10]; therefore, the possible relationship between food hypersensitivity against beef and vaccine-related allergic reactions in dogs should be investigated.

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