Effects of routine prophylactic vaccination or administration of aluminum adjuvant alone on allergen-specific serum IgE and IgG responses in allergic dogs

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Objective—To determine the acute corn-specific serum IgE and IgG, total serum IgE, and clinical responses to SC administration of prophylactic vaccines and aluminum adjuvant in corn-allergic dogs.

Animals—20 allergic and 8 nonallergic dogs.

Procedure—17 corn-allergic dogs were vaccinated. Eight clinically normal dogs also were vaccinated as a control group. Serum corn-specific IgE, corn-specific IgG, and total IgE concentrations were measured in each dog before vaccination and 1 and 3 weeks after vaccination by use of an ELISA. The corn-allergic dogs also had serum immunoglobulin concentrations measured at 8 and 9 weeks after vaccination. Twenty allergic dogs received a SC injection of aluminum adjuvant, and serum immunoglobulin concentrations were measured in each dog 1, 2, 3, 4, and 8 weeks after injection. The allergic dogs were examined during the 8 weeks after aluminum administration for clinical signs of allergic disease.

Results—The allergic dogs had significant increases in serum corn-specific IgE and IgG concentrations 1 and 3 weeks after vaccination but not 8 or 9 weeks after vaccination. Control dogs did not have a significant change in serum immunoglobulin concentrations after vaccination. After injection of aluminum adjuvant, the allergic dogs did not have a significant change in serum immunoglobulin concentrations or clinical signs.

Conclusions and Clinical Relevance—Allergen-specific IgE and IgG concentrations increase after prophylactic vaccination in allergic dogs but not in clinically normal dogs. Prophylactic vaccination of dogs with food allergies may affect results of serologic allergen-specific immunoglobulin testing performed within 8 weeks after vaccination. (*Am J Vet Res* 2005;66:1572–1577)

Allergic disease is a major problem in humans and dogs. In people, it is estimated that the prevalence of atopic disease in developed countries exceeds 30%.[1] Furthermore, the incidence of allergic diseases in general, especially in children, has increased during recent decades in many countries.[1-3] In dogs, the prevalence and incidence of allergic disease are unknown; however, it is suspected that atopic dermatitis affects up to 10% of the canine population.[4] In a retrospective study[5] of dogs examined at a referral veterinary dermatology practice, 19 of 251 (7.6%) dogs had an underlying food allergy.

It is believed that most cases of allergic disease in humans are mediated by IgE.[6,7] There is also evidence for the role of IgE in allergic diseases (eg, atopic dermatitis or food allergy) of dogs.[8] Furthermore, a significant and predictable increase in serum allergen-specific IgE concentrations has been documented[9] after oral challenge with dietary allergens in Maltese-Beagle crossbred dogs with naturally developing food allergies. An IgG response often accompanies an IgE response to allergens in dogs with atopic dermatitis,[10] although IgG is not currently considered to have a pathogenic role in allergic diseases of dogs.[8]

Multiple prophylactic vaccines are recommended for people and dogs during the immature and adult years. In people and dogs, prophylactic vaccination leads to the induction of IgE and IgG expression. In children, administration of tetanus-diphtheria toxoid vaccine without an aluminum adjuvant results in the production of tetanus-diphtheria toxoid–specific IgE and IgG responses.[11] In dogs, administration of a prophylactic multivalent viral vaccine containing distemper virus results in an increase in IgE antibody response to pollens to which the dogs have been sensitized[12] and an increase in IgG antibody response.[13,14]

Some prophylactic vaccines contain aluminum adjuvants.[15,16] Aluminum adjuvants enhance the immune response in humans via the induction of cytokines, such as interleukin-4, -5, -6, -9, -10, and -13 from T–helper-2 lymphocytes (type 2 response).[16,17] There is concern that some prophylactic vaccines, with or without aluminum adjuvants, may influence the development of allergic disease to vaccine antigens in humans.[18-20] Repeated SC administration of food or pollen antigen extracts in combination with aluminum to dogs has been used to induce allergic-type diseases in dogs.[21,22]

In another study,[23] investigators evaluated the effect of prophylactic vaccination on responses of total serum IgE and IgE specific to vaccine components during a 4-year period in nonallergic Beagles. They detected IgE responses in the dogs 2 weeks after vaccination with a prophylactic rabies vaccine containing an aluminum adjuvant. In Beagle puppies, administration of a series of prophylactic vaccines, one of which contained an aluminum adjuvant, resulted in the production of IgG antibodies against various bovine, murine, and porcine antigens.[24]

At our research facility, there exists a unique colony of Maltese-Beagle crossbred dogs with naturally developing allergic disease. These dogs characteristically have an IgE-mediated food hypersensitivity to corn and soy and a significant and predictable increase in serum allergen-specific IgE concentrations after oral challenge with dietary allergens.[9,25]

To our knowledge, the possibility that vaccination may increase concentrations of IgE specific for previously sensitized allergens in dogs with naturally developing allergies has not been investigated. The purpose of the study reported here was to measure serum allergen-specific antibody responses after SC administration of prophylactic vaccines and examine the clinical and antibody responses after SC administration of an aluminum adjuvant alone in dogs with naturally developing allergies in a controlled setting.

Materials and Methods

Animals—Twenty allergic Maltese-Beagle crossbred dogs from our research colony and 8 nonallergic mixed-breed dogs were used in the study. All Maltese-Beagle crossbred dogs were maintained on a commercial diet that did not contain corn or soy,[a] thereby preventing the manifestation of allergic signs secondary to food allergy. All mixed-breed dogs were maintained on a standard commercial laboratory ration.[b] The dogs were housed in groups at our laboratory research facility. The day-night cycle was 12 hours of light and 12 hours of darkness. All experiments were approved by an institutional animal care and use committee.

Experiment 1—An experiment was conducted to determine the serum immunologic effect of SC administration of prophylactic vaccines in allergic and nonallergic dogs. In general, the prophylactic vaccination schedule for most laboratory dogs at our research facility is in accordance with our university canine vaccination guidelines.[26] Briefly, 5-week-old dogs receive a canine distemper and human measles vaccine.[c] The dogs then receive a multivalent canine distemper virus, adenovirus-2, parvovirus, and parainfluenza virus vaccine[d] every 3 to 4 weeks until they are 17 weeks old. An initial rabies vaccination is administered when dogs are 16 weeks old and then again within 1 year. Subsequently, the multivalent vaccine is administered every year, and the rabies vaccination is administered every 3 years.

Seventeen allergic Maltese-Beagle crossbred dogs and 8 nonallergic mixed-breed dogs were used in experiment 1. All dogs involved in experiment 1 were scheduled for their yearly prophylactic vaccination. Maltese-Beagle dogs ranged from 1.9 to 5.8 years of age (mean, 4.5 years) and weighed between 4.6 and 7.8 kg (mean, 6.0 kg). The group consisted of 6 sexually intact females, 4 spayed females, and 7 sexually intact males. Mixed-breed nonallergic dogs ranged from 1 to 6 years of age (mean, 2.7 years) and weighed 17.9 to 30 kg (mean, 24.1 kg). The group consisted of 6 sexually intact females and 2 sexually intact males.

A blood sample (4 to 6 mL) was collected from each dog by jugular venipuncture. This sample was collected 3 weeks before the annual vaccination for the allergic dogs and on the day of vaccination for the nonallergic dogs. Each blood sample was centrifuged and the serum separated and frozen at –70°C. On the first day of the study (day 0), a physical examination was performed on each dog and each dog was then vaccinated. All 17 allergic and 8 nonallergic dogs were vaccinated by SC administration of the multivalent prophylactic vaccine[d] containing attenuated strains of canine distemper virus, canine adenovirus-2, canine parainfluenza virus, and canine parvovirus propagated on an established canine cell line. Ten allergic dogs and 1 nonallergic dog were also vaccinated by SC administration of a monovalent, killed rabies vaccine containing an aqueous, nonaluminum adjuvant.[e] Neither the multivalent vaccine nor the rabies vaccine contained corn, soy, or any added aluminum adjuvant.[f,g] Serum samples were obtained from all dogs 1 and 3 weeks after vaccination. Serum samples were also obtained from the allergic dogs 8 and 9 weeks after vaccination. For comparison, weekly serum samples were also obtained from the allergic dogs during a 3-week period when they had not been recently vaccinated. This 3-week period was within the same season during which experiment 2 was conducted.

Experiment 2—A controlled crossover study was performed to determine the effect of SC administration of aluminum alone. Twenty allergic dogs from the aforementioned research colony were randomly assigned to group A (control group) or B (aluminum treatment). Seventeen of these dogs had been used in experiment 1. In experiment 2, allergic dogs ranged from 2.8 to 6.4 years of age (mean, 5.0 years) and weighed between 4.5 and 7.5 kg (mean, 5.9 kg). The allergic dogs consisted of 7 sexually intact females, 6 spayed females, and 7 sexually intact males. Experiment 2 was initiated 300 days after the administration of the prophylactic vaccines in experiment 1. The yearly annual prophylactic vaccinations for the dogs in this experiment were delayed until after the completion of the experiment.

For experiment 2, a serum sample was obtained and physical examination was performed on each dog (day 0). Because aluminum adjuvants in prophylactic vaccines are aluminum salts (ie, aluminum hydroxide, aluminum phosphate, or potassium aluminum sulfate[16]), dogs of group B received a SC injection of aluminum hydroxide containing 0.85 mg of aluminum. The amount of aluminum in this injection was equivalent to the maximum amount of aluminum (ie, 0.85 mg) allowed in vaccines licensed for use in human infants.[15] Similar standards do not exist for canine vaccines.[h] Dogs of group A received a SC injection of an equal volume of sterile saline (0.9% NaCl) solution. The investigators were unaware of the injections administered to each dog.

At 7-day intervals throughout the experiment, a blood sample (4 to 6 mL) was collected from each dog via jugular venipuncture. The blood sample for each dog was processed as described for experiment 1. Eight weeks after the start of the study (day 56), the treatment for each group of dogs was switched and dogs of group A received a SC injection of aluminum hydroxide, whereas dogs of group B received a SC injection of saline solution. We selected an interval of 8 weeks after administration of the aluminum adjuvant before administration of the second injection on the basis that immunologic changes observed in experiment 1 had returned to prevaccination values at this point.

In experiment 2, the clinical response to SC administration of aluminum to allergic dogs was evaluated through clinical scores of skin lesions and pruritus scores. Clinical scores of skin lesions assessed 3 criteria (erythema, excoriations, and number of papules and pustules).[9] Each of these criteria is graded on a scale of 0 to 3 (0 represented normal skin, and 3 represented severely affected skin). Thirty-five areas of the skin (including the ear canal) were examined on each dog; therefore, a maximum score of 315 can be achieved. All clinical examinations of skin lesions were performed by the same investigator (KCT). Scores of previous clinical examinations of skin lesions for each dog were not provided to the investigator at the time of subsequent examinations.

Each dog was also observed for evidence of pruritus, and a pruritus score was assigned on a scale of 0 to 5 (0 represented no pruritus, and 5 represented constant scratching). All pruritus scores were assigned by the same investigator (JP).

Investigators were not aware of the treatment group of each dog.

During the first 3 weeks after injection, clinical scores of skin lesions were determined 3 d/wk and pruritus scores were determined 5 d/wk. After that initial 3-week period, clinical scores of skin lesions were determined 1 d/wk and pruritus scores were determined 3 d/wk. We chose to perform more intense observation during the first 3 weeks after injection because these allergic Maltese-Beagle crossbred dogs typically have a clinical response to allergen challenge during this time frame.[25]

Measurement of total serum IgE concentration—Total serum IgE concentration was measured by use of an ELISA. Mouse monoclonal 5.91 anti-dog IgE[27] (10 µg/mL) diluted in 0.1M carbonate bicarbonate buffer (pH, 9.5) was used to coat microtiter styrene U-bottom plates[i] (50 µL/well) by incubation overnight at 4°C. Each plate was washed 3 times with **PBS solution containing 0.05% Tween 20 (PBST)**; then plates were blocked by the addition of 200 µL of 1% dry milk in PBS solution/well (experiment 1) and 200 µL of PBST/well[28] (experiment 2), and plates were incubated for 2 hours at 25°C. Blocked plates were washed once, and serum samples (50 µL/well) were added to each plate. Each serum sample was diluted 1:5 and analyzed in triplicate. After addition of samples, plates were incubated for 2 hours. Plates were then washed 5 times, and biotinylated mouse monoclonal 5.91 anti-dog IgE[27] (1 µg/mL) in PBST containing 10% normal mouse serum and 10 µg of dog IgG[j]/mL were added to each well. Plates were incubated for 1 hour and then washed 5 times with PBST before the addition of streptavidin peroxidase[k] (diluted 1:1,000) followed by incubation for another 30 minutes. Plates were washed 5 times, and color was developed by adding substrate.[l] Plates were analyzed at 450 nm on a microtiter plate reader.[m] A standard curve consisting of serial dilutions of absolute quantities of monoclonal canine IgE[29] allowed for absolute measurement of total serum IgE.

Measurement of corn-specific serum IgE and IgG concentrations—Corn-specific IgE and IgG concentrations were evaluated by use of an ELISA because the Maltese-Beagle crossbred dogs have been characterized with a naturally developing IgE-mediated food hypersensitivity to corn and soy.[9,25] Prior to coating the plates, a corn allergen protein preparation[n] was dialyzed in PBS solution (pH, 7.4) to remove glycerin. Linear epitopes were revealed by boiling for 5 minutes after the addition of 5% 2-mercaptoethanol. After centrifugation, the denatured corn protein solution was dialyzed against PBS solution, adjusted to a concentration of 1 mg/mL, diluted to 50 µg/mL in 0.1M carbonate bicarbonate buffer (pH, 9.5), and used to coat the plates (50 µL/well) before incubation overnight at 4°C. Detection of corn-specific IgE was accomplished by use of a biotinylated mouse monoclonal anti-dog 5.91 IgE[27] in PBST containing 10% normal mouse serum and 10 µg of dog IgG[j]/mL, as described previously. For the corn-specific IgG, peroxidase-labeled γ-chain–specific goat anti-dog IgG[o] (2 µg/mL) was added to each plate and plates were then incubated for 1 hour. Concentrations of corn-specific IgE and IgG were determined in relation to a standard curve developed from a serum sample that contained high amounts of corn-specific IgE and IgG and was serially diluted for inclusion on each plate. Optical density readings for corn-specific IgE in the serum sample that was used to develop the standard curve ranged from 0.138 to 0.262 when used at a dilution of 1:10, whereas optical density readings for corn-specific IgG in the serum sample used to develop the standard curve ranged from 0.103 to 0.181 when used at a dilution of 1:50.

Statistical analyses—Statistical analyses were performed by use of commercial software.[p] For experiment 1, a 1-way, repeated-measures ANOVA followed by the Bonferroni post hoc test was used to determine differences between serum immunoglobulin concentrations before and after vaccination. A 2-way, repeated-measures ANOVA was used to determine the effect of sex, age, and vaccines received on immunoglobulin concentrations before and after vaccination. For experiment 2, a 1-way, repeated-measures ANOVA followed by the Bonferroni post hoc test was used to determine differences between serum immunoglobulin concentrations, clinical scores of skin lesions, and pruritus scores before and after injection of aluminum or saline solution. A 2-way, repeated-measures ANOVA was used to determine the effect of sex and age on immunoglobulin concentrations before and after injection of aluminum or saline solution.

Results

Experiment 1—Immunologic effects of SC administration of prophylactic vaccine were measured in allergic and nonallergic dogs. In the allergic Maltese-Beagle crossbred dogs, a significant increase in serum concentrations of corn-specific IgE and IgG was observed 1 week ($P < 0.001$ for IgE and $P = 0.01$ for IgG) and 3 weeks ($P < 0.001$ for IgE and IgG) after vaccination but not 8 or 9 weeks after vaccination (**Figure 1**). A significant increase in serum total IgE concentration was not observed after vaccination in the allergic
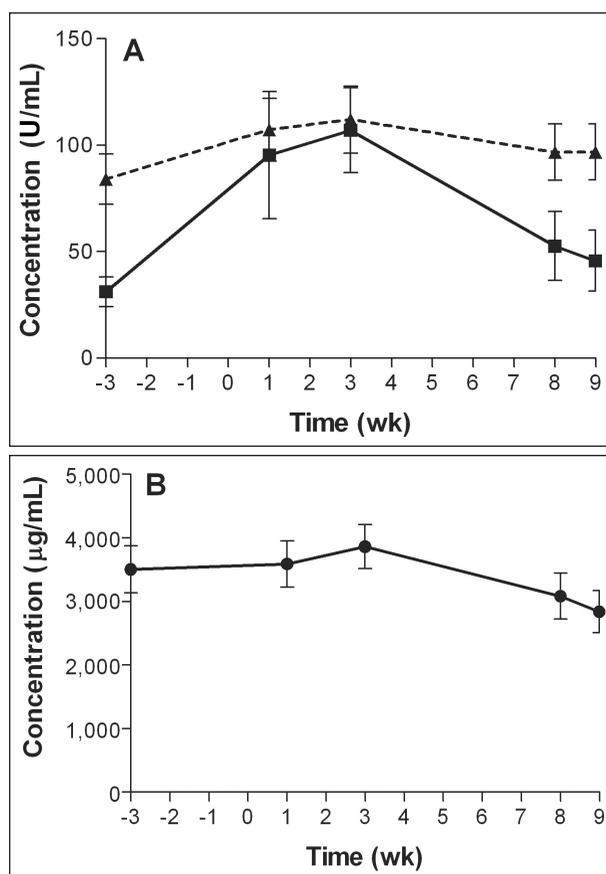

Figure 1—Mean ± SEM serum concentrations of corn-specific IgE (squares) and corn-specific IgG (triangles; A) and total IgE (B) in 17 allergic Maltese-Beagle crossbred dogs after prophylactic vaccination. Administration of prophylactic vaccines was designated as week 0. Serum samples for week 0 were obtained before administration of prophylactic vaccines.
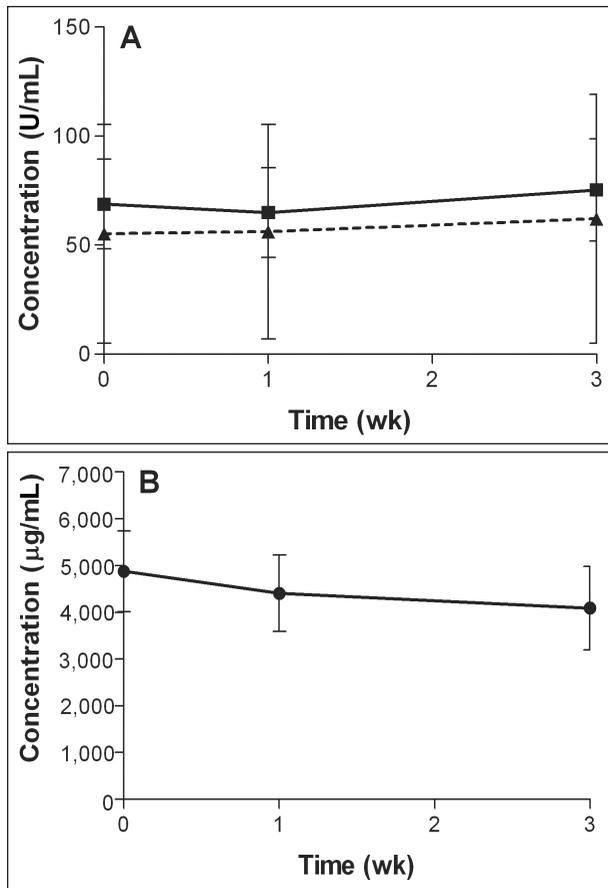
Figure 2—Mean ± SEM serum concentrations of corn-specific IgE (squares) and corn-specific IgG (triangles; A) and total IgE (B) in 8 nonallergic mixed-breed dogs after prophylactic vaccination. Administration of prophylactic vaccines was designated as week 0. Serum samples for week 0 were obtained before administration of prophylactic vaccines.

dogs. There were no changes in serum concentration of corn-specific IgE, corn-specific IgG, or total IgE in the allergic dogs during the 3-week period when they had not been recently vaccinated.

The clinically normal, mixed-breed, nonallergic (control) dogs did not have a significant change in serum concentration of corn-specific IgE, corn-specific IgG, or total IgE after vaccination (**Figure 2**). There was no significant effect of age, sex, or vaccine administered on the serum immunoglobulin concentrations for the allergic and nonallergic dogs after vaccination.

Experiment 2—Clinical and immunologic effects of SC administration of aluminum adjuvant to allergic dogs were evaluated. There was no significant difference in the clinical scores for skin lesions or pruritus scores of the allergic dogs after administration of aluminum adjuvant or saline solution. We did not detect significant differences in serum concentrations of corn-specific IgE, corn-specific IgG, and total IgE after the administration of aluminum adjuvant. Furthermore, we did not detect a significant effect of age or sex on serum immunoglobulin concentrations.

Discussion

Analysis of results of the study reported here indi-cated that prophylactic vaccination of dogs with natu-rally developing allergies causes a transient but signifi-cant increase in allergen-specific IgE and IgG concen-trations. This increase in allergen-specific immunoglobulin concentrations was not detected when clinically normal dogs without allergies were vaccinated. This increase in allergen-specific immunoglobulin concentrations also was not detected when the allergic dogs received a SC injection of alu-minum, a vaccine adjuvant.

An explanation for the lack of an immunologic effect after administration of an aluminum adjuvant is that the amount of aluminum hydroxide administered in the study may have been too low to elicit an immunologic effect. The amount of aluminum (ie, 0.85 mg) adminis-tered SC to the allergic dogs was chosen because it rep-resented the maximum amount of aluminum allowed in prophylactic vaccines for human infants.[15] The amount of aluminum in veterinary vaccines is unknown because it is considered proprietary informa-tion by vaccine manufacturers, although various researchers have assayed the amount of aluminum in some veterinary vaccines. A rabies vaccine[q] containing an aluminum adjuvant that caused an increase in immunoglobulin concentrations after vaccination of nonallergic dogs was determined to have an aluminum content of 32.6 µg/g.[23] However, the number of vials or lots of vaccine assayed to determine the aluminum content in that study was not reported, and it is uncer-tain whether this is an accurate representation of the amount of aluminum contained in all doses of this rabies vaccine. In another study[30] from 1997, investiga-tors reported that the amount of aluminum in vaccines intended for use in small animals varied between 350 µg/g for some FeLV vaccines to almost 800 µg/g for some 1-year rabies vaccines. The number of vaccines assayed and method used for the assays in that study were not reported. Despite these reported aluminum concentrations, the minimum and maximum alu-minum content of currently used veterinary vaccines is in general not known.

The increase in allergen-specific immunoglobulin concentrations detected in the food-allergic dogs after vaccination was not caused by an aluminum adjuvant. Although the exact amount of aluminum in the multi-valent and rabies vaccines used in the study reported here was not known, neither vaccine contained an alu-minum adjuvant; thus, it was assumed that the amount of aluminum, if any, in the vaccines would be small. The aluminum content of another multivalent vaccine[r] made by the same manufacturer has been analyzed[23] and reportedly contains only 0.06 µg/ of aluminum/g. The allergic dogs in experiment 2 received 0.85 mg/g of aluminum SC. The lack of an immunologic response to an amount of aluminum that exceeded the trace amounts of aluminum detected in a similar multivalent vaccine supports the hypothesis that the increases in allergen-specific immunoglobulin concentrations after vaccination were not caused by an aluminum adjuvant.

Naturally acquired viral infections in humans can lead to the induction of IgE and IgG expression,[31-33] and viral infection of human B lymphocytes causes the lymphocytes to switch from IgM to IgE production.[34,35]

Incubation of human B lymphocytes with a live, attenuated, measles-mumps-rubella vaccine induces the expression of IgE mRNA through the activation of an antiviral protein kinase.[36] Because canine distemper virus and human measles virus are members of the genus *Morbillivirus* of the family Paramyxoviridae, we hypothesized that the increase in allergen-specific immunoglobulin concentrations detected in the allergic dogs after vaccination in our study was the result of live, attenuated virus (especially distemper virus) in the multivalent vaccine.

We did not detect changes in serum total IgE concentrations after prophylactic vaccination of the allergic dogs in experiment 1. Vaccination of clinically normal dogs with an aluminum adjuvant containing rabies vaccine[r] resulted in increased serum total IgE concentrations 2 weeks after vaccination.[23] However, an increase in serum total IgE concentration has not been detected in the food-allergic Maltese-Beagle crossbred dogs after oral challenge with allergens. Other studies[9,25] conducted with the food-allergic dogs used in the study reported here revealed significant and predictable increases in serum allergen-specific IgE concentrations but not total IgE concentrations after oral challenge with dietary allergens. Multiple factors other than allergic disease or vaccination can influence serum total IgE concentrations in a dog[37]; therefore, we do not recommend the use of serum total IgE concentrations for the diagnosis of allergic disease in dogs.

It is unknown whether the results of our study could have been affected by an anamnestic response to the vaccines. In another study,[23] a greater increase in serum total IgE concentrations was detected after vaccination in clinically normal dogs as they grew older and received a greater number of vaccinations. The allergic and clinically normal dogs in the study reported here were of various ages. Although the complete vaccination history of all allergic and clinically normal dogs was not known, it is logical to assume that an older dog would typically have received a larger total number of vaccinations in its lifetime. Because there was no effect of age on the immunoglobulin concentrations in the dogs after vaccination, we believed that there was no cumulative effect of vaccinations on the immunoglobulin concentration.

It is unknown whether the acute increase in allergen-specific immunoglobulin concentrations after administration of a prophylactic vaccine in the allergic dogs was accompanied by clinical signs consistent with an allergic response, such as pruritus or erythema. Clinical response of the allergic dogs was not monitored in experiment 1. It is unknown whether administration of a prophylactic vaccine in dogs with clinical signs of allergies would cause an exacerbation of the clinical signs of allergic disease.

Observation of an acute increase in allergen-specific immunoglobulin concentrations after administration of a prophylactic vaccine has important clinical implications in the diagnosis of allergic disease in dogs. Serum-based in vitro allergy tests are commercially available to veterinarians and are used in the diagnostic evaluation of dogs with allergies. These laboratory tests consist of panels of allergen-specific IgE measurements reported on a semiquantitative scale. The detection of an allergen-specific IgE concentration greater than a certain value or score provided by the manufacturer of the allergy test is used to support the diagnosis of an allergy to that allergen. Because results of the study reported here document that an increase in allergen-specific IgE concentration to allergens to which a dog has previously been sensitized can be seen after vaccination, it is possible that administration of prophylactic vaccines during the 8-week period preceding testing by use of serologic allergen-specific IgE panels could result in erroneous interpretation of results of serologic allergy tests in food-allergic dogs.

The increase in allergen-specific immunoglobulin concentrations was evident by 1 week after vaccination and persisted until 3 weeks after vaccination. Allergen-specific immunoglobulin concentrations returned to prevaccination values by 8 weeks after vaccination. It is unknown whether the increase in allergen-specific immunoglobulin concentrations resolved before 8 weeks after vaccination because serum immunoglobulin concentrations were not measured between weeks 4 and 7 after vaccination.

Prophylactic vaccination of allergic dogs results in an acute increase in serum concentrations of immunoglobulins specific to allergens to which the dogs had previously been sensitized. This increase in allergen-specific immunoglobulin concentrations was evident within 1 week after vaccination but resolved by 8 weeks after vaccination. The SC administration of 0.85 mg of aluminum to allergic dogs does not cause a significant acute change in immunoglobulin concentrations or clinical signs of allergic disease. Administration of prophylactic vaccines could increase allergen-specific IgE concentrations measured by serologic allergen-specific IgE allergy test panels when the vaccinations are administered within 8 weeks before testing is conducted in food-allergic dogs.

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