

Effect of vaccination on serum concentrations of total and antigen-specific immunoglobulin E in dogs

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Objective—To determine the effect of vaccination on serum concentrations of total and antigen-specific IgE in dogs.

Animals—20 female Beagles.

Procedure—Groups of 5 dogs each were vaccinated repeatedly between 8 weeks and 4 years of age with a multivalent and rabies vaccine, a multivalent vaccine only, or a rabies vaccine only. A fourth group of 5 dogs served as unvaccinated controls. Serum concentrations of total immunoglobulins and antigen-specific IgE were determined following vaccination.

Results—The multivalent vaccine had little effect on serum total IgE concentrations. The concentration of IgE increased slightly following vaccination for rabies at 16 weeks and 1 year of age and increased greatly after vaccination at 2 and 3 years of age in most dogs, with a distinct variation between individual dogs. Vaccination had no effect on serum concentrations of IgA, IgG, and IgM as measured at 2 and 3 years of age. The rabies vaccine contained aluminum adjuvant in contrast to the multivalent vaccine. An increase of IgE that was reactive with vaccine antigens, including bovine serum albumin and bovine fibronectin, was detected in some of the dogs vaccinated for rabies. There was no significant correlation between serum concentrations of total IgE and antigen-specific IgE following vaccination. Serum total IgE concentration rapidly returned to preimmunization concentrations in most dogs, but high concentrations of antigen-specific IgE persisted.

Conclusions and Clinical Relevance—Vaccination of dogs for rabies increases serum concentrations of total IgE and induces IgE specific for vaccine antigens, including tissue culture residues. Vaccination history should be considered in the interpretation of serum total IgE concentrations. (*Am J Vet Res* 2002;63:611–616)

High serum IgE concentrations in humans are associated with allergic diseases such as asthma, atopic dermatitis, and allergic rhinitis, and quantification of total IgE is used as a diagnostic aid in the assessment of patients with allergic disease.^{1,2} For humans, inter-

pretation of serum total IgE concentration should take into account the influence of factors other than allergic diseases, including age, sex, and smoking.^{3,4} It is well known that immunization of humans with vaccines that contain either aluminum salts or pertussis toxin as adjuvants can induce antigen-specific IgE,^{5,8} and this may occasionally cause local or systemic allergic reactions.⁸ However, the effect of vaccination on serum total IgE concentration has not been investigated in detail. In one study, vaccination with aluminum-adsorbed tetanus toxoid greatly increased the serum total IgE concentration in 5 of 22 individuals.⁶ In another study, a modest, but insignificant, increase of serum total IgE concentration following vaccination was observed.⁷

Dogs spontaneously develop allergies with atopic dermatitis and food allergy as the most common clinical manifestations. Spontaneous and experimentally induced allergic disease in dogs have been proposed as models of allergic disease in humans.^{9,10} The presence of IgE bound to mast and Langerhans cells in the skin of atopic dogs^{11,12} suggests that IgE plays a similar role in the pathogenesis of allergic disease in dogs as in humans. However, the measurement of serum total IgE concentration has not yet proven to be useful in the diagnosis of allergic disease.¹³ The IgE concentration is influenced by the genetic background, age, sex, and unidentified environmental factors.^{9,14,15} The effect of vaccination on the serum IgE concentration in dogs has not been determined. Results of one study suggested that vaccination facilitated the experimental induction of pollen-specific IgE and atopic dermatitis,¹⁶ but the mechanism and the serum total IgE concentration were not determined. In the study presented here, we report the effect of vaccination on the serum concentrations of total immunoglobulins and on IgE that is reactive with vaccine antigens in dogs. Vaccines may contain bovine serum components either as tissue culture residues or added as stabilizers.^{17,20} Therefore, we also determined whether vaccination induced IgE that is reactive with bovine serum albumin (BSA) and bovine fibronectin (BFN).

Materials and Methods

Animals—Twenty female Beagles were obtained from 5 litters and allocated to 4 experimental groups of 5 dogs each according to litter of origin and body weight at 8 weeks of age. Dogs were housed indoors with 2 or 3 dogs per run and fed commercial chow and water ad libitum. The day-light cycle was 12 hours. Dogs were housed in 2 separate rooms according to the vaccination strategy and maintained under National Institutes of Health guidelines for animal use and

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care. The study was approved by the Purdue University Animal Care and Use Committee. All dogs remained clinically healthy during the course of our study.

Vaccination schedule and serum collection—Dogs were vaccinated subcutaneously with a multivalent^a and rabies vaccine^b (MR-group dogs), with the multivalent vaccine only (M-group dogs), or with the rabies vaccine only (R-group dogs). Dogs in the control group were injected subcutaneously with 1 ml of sterile saline (0.9% NaCl) solution following the same schedule as MR-group dogs.

The multivalent vaccine was administered at 8, 10, 12, 16, 20, 26, and 52 weeks of age and every 6 months thereafter. The rabies vaccine was administered at 16 and 52 weeks of age and then once a year. Serum was collected from dogs at 8, 16, and 26 weeks of age and then before vaccination and 2 weeks after vaccination. Additional serum samples were collected at 2, 6, 10, and 14 weeks after vaccination of dogs at 4 years of age.

Viral serology—The titers of antibodies against canine distemper virus and canine parvovirus-2 were determined by a serum neutralization test^c and hemagglutination inhibition test,^c respectively. Titers $\geq 1:32$ and $\geq 1:80$, respectively, are considered protective. The antibody titer against rabies virus was determined by a rapid fluorescent focus inhibition test.^d A titer of 1:5 and higher is considered protective.

Total immunoglobulin quantitation in serum—Serum concentrations of IgA, IgG, and IgM were determined by ELISA. Polystyrene microtiter plates were coated overnight at 4 C with affinity purified goat-anti dog IgA, IgG, or IgM^e in 0.1M carbonate buffer (pH 9.6). Standards were prepared by appropriate dilutions of a reference serum^e in phosphate buffered saline solution (PBSS) and 0.05% Tween, and serum samples were diluted 1:1,000, 1:10,000, and 1:100,000 in PBSS solution and 0.05% Tween. Standards and serum samples were added to the wells and incubated for 2 hours at 37 C. Bound canine immunoglobulins were detected with alkaline phosphatase labeled anti-dog IgA, IgG, or IgM,^e and enzyme reactivity was revealed with 1 mg of p-nitrophenyl phosphate/ml. The plate was read at 405 nm in a microplate reader.^f

Total concentration of IgE in the serum samples was measured by use of a sandwich-type monoclonal antibody-based ELISA, as previously described.²¹ Briefly, diluted serum samples were added to anti-canine IgE-coated polystyrene microplate wells. After overnight incubation at 4 C, wells were washed and incubated with biotinylated anti-canine IgE, followed by avidin-alkaline phosphatase, then p-nitrophenyl phosphate substrate. Color development was determined by use of a microplate reader at 405 nm. Samples were screened at a dilution of 1:5 and retested at higher dilutions of up to 1:1,000 as necessary. Each plate included serial dilutions of an internal reference standard (the American Academy of Veterinary Dermatology canine IgE reference standard, consisting of pooled canine serum from atopic donors) arbitrarily assigned a concentration of 100 U/ml. A standard curve prepared from these serial dilutions was used to calculate the IgE concentration in the unknown serum samples in U/ml. Under these assay conditions, 1 U/ml is equivalent to approximately 0.23 μg of IgE/ml.²¹

Measurement of antigen-specific IgE—The multivalent vaccine was supplied as 1 vial (virus component) containing the lyophilized viruses and 1 vial (diluent) containing the diluent and leptospira bacterin. Microtiter plates were coated overnight with either the virus component dissolved in sterile saline solution and diluted 1:10 with carbonate buffer (final pH, 9.33), with the diluent diluted 1:10

in carbonate buffer (final pH, 9.36), or with the rabies vaccine diluted 1:10 in carbonate buffer (final pH, 9.37). Plates were washed and incubated for 1 hour with PBSS solution and 0.05% Tween, followed by a 2 hour incubation with serum, diluted 1:10 in PBSS solution and Tween, at 37 C. Bound immunoglobulin was detected with monoclonal anti-dog IgE (clone D9, 1:400 of ascites fluid²²), followed by alkaline phosphatase-labeled anti-mouse IgG.^g The substrate was p-nitrophenyl phosphate, and the plate was read at 405 nm. Optical densities ≥ 0.1 were considered biologically important.

In other experiments, microtiter plates were coated with BSA or BFN (10 $\mu\text{g}/\text{ml}$). Plates were washed and incubated for 1 hour at 37 C with PBSS solution and Tween, followed by incubation with 1:10 diluted serum samples. The binding of IgE was detected with the monoclonal antibody as already described. The binding of IgG was determined with alkaline phosphatase-labeled goat anti-dog IgG^c as previously described.²⁰ Optical densities ≥ 0.1 were considered biologically important.

Measurement of the aluminum content of the vaccines—Contents of the multivalent vaccine, the rabies vaccine, and tissue culture medium (RPMI-1640 with 10% fetal calf serum) were analyzed for aluminum content by inductively coupled plasma (ICP) spectrometry. Three hundred microliters of the vaccine was mixed with 5 ml of concentrated nitric acid and heated briefly to 175 C until a brown gas was eluted, indicating the beginning of the reaction. Samples were cooled for 30 minutes, then 30% hydrogen peroxide was added (4 ml), and samples were heated to 175 C until the volume was reduced to approximately 1 to 1.5 ml. After cooling, the samples were brought to volume in a 10-ml volumetric flask. Measurements were performed in a ICP spectrometer.^h

Statistical analysis—Significant differences among groups in serum concentrations of total IgA, IgG, and IgM were determined by a 1-way ANOVA with $P < 0.05$. Serum total IgE concentration was not normally distributed and analyzed with a Mann-Whitney U test with $P < 0.05$. A Pearson correlation coefficient was used to determine the relationship between serum concentrations of antigen-specific and total IgE. Relationships were considered significant at $P < 0.05$.

Results

The M- and MR-group dogs were vaccinated 5 times as puppies and then at 6-month intervals with the multivalent vaccines. Such a protocol is rarely used in pet dogs and occasionally in kennels, and it was adopted for our study to determine whether such an intensive protocol would cause any changes in several immune variables. As expected, all MR- and M-group dogs developed high antibody titers against distemper and parvovirus as determined by serum neutralization and hemagglutination inhibition tests, respectively (Fig 1).

The MR- and R-group dogs were injected with the rabies vaccine at 16 weeks of age and then annually. This protocol is commonly practiced, and law in several states in the United States, including Indiana where our study was conducted, mandates annual vaccination for rabies. The MR- and R-group dogs developed protective titers against rabies as determined by the rapid fluorescence focus inhibition assay (Fig 1).

Serum concentrations of total IgA, IgG, and IgM were measured at 2 and 3 years of age following the

annual vaccination. There was distinct individual variation in the concentration of IgA (range, 0.55 to 6.47 mg/ml), IgG (6.2 to 35.8 mg/ml), and IgM (0.27 to 4.31 mg/ml), but there were no significant differences among the 4 groups of dogs at 2 (not shown) and 3 years of age (Fig 2). In contrast, the concentration of IgE varied greatly among the groups of dogs. The concentration of IgE in the control dogs remained low at all time points, and at 158 weeks the concentration varied from 0.2 to 2.2 U/ml (Fig 3). The serum IgE concentration in M-group dogs was only slightly, although significantly, higher and varied between 1.2 and 10.5 U/ml. Although there was distinct variability in IgE concentrations in MR-group (46.0 to 488.6 U/ml) and R-group (8.8 to 966.7 U/ml) dogs, the IgE concentrations were significantly increased in comparison with control dogs.

The serum IgG concentration increased after each vaccination, but there was a much greater increase after vaccination at 2 and 3 years of age in comparison to vaccinations at 16 weeks, 1 year, and 4 years of age (Fig 4). The high concentration of IgE was transient and returned to the low concentration observed prior to the annual vaccination for rabies.

Specificity of IgE antibodies for vaccine antigens was determined. There were no detectable IgE antibodies against vaccine components in R-group dogs and low amounts in M-group dogs. Four dogs in the MR group and 5 dogs in the R group had IgE antibodies that were reactive with the rabies vaccine, and 3 dogs in the MR group and 4 dogs in the R group had IgE antibodies reactive with the diluent component of the multivalent vaccine. None of the dogs had IgE antibodies reactive with the virus component of the multivalent vaccine (Table 1). There was no significant correlation between the serum total IgE concentration and the IgE reactivity against vaccine components (total IgE vs rabies-specific IgE, $r = 0.252$, $P = 0.283$; total IgE vs diluent-specific IgE, $r = 0.291$, $P = 0.213$). The rabies- and diluent-specific IgE, however, were significantly correlated ($r = 0.665$, $P = 0.001$).

To determine whether the vaccination induced antibodies against bovine serum components, the presence of BSA- and BFN-specific IgG and IgE was measured. All vaccinated dogs had high concentrations of BSA- and BFN-specific IgG (Table 1). Six dogs that were vaccinated for rabies had high concentrations of BSA-specific IgE, and 3 dogs had increased BFN-specific IgE (Table 1); however, there was no correlation between the total IgE concentration and BSA-specific IgE ($r = 0.260$, $P = 0.269$) or BFN-specific IgE ($r = 0.229$, $P = 0.332$). The concen-

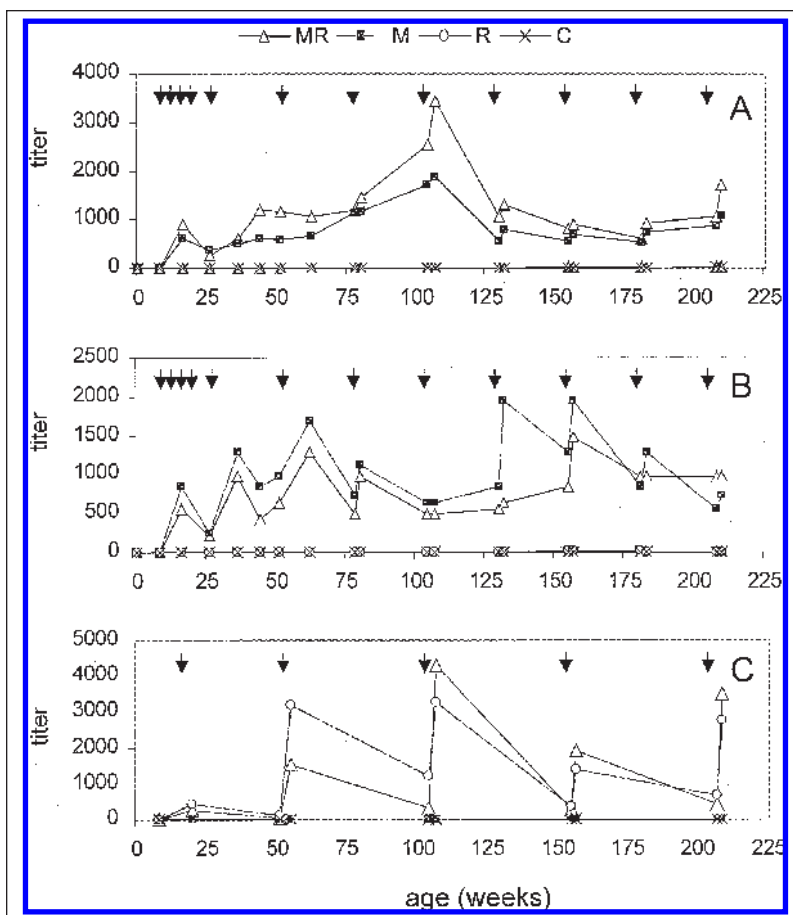


Figure 1—Time course of geometric mean titers of antibodies against canine distemper virus (A—neutralization test), canine parvovirus-2 (B—hemagglutination inhibition test), and rabies virus (C—rapid focus inhibition assay). Arrows indicate the times at which dogs received the multivalent vaccine (A and B) and the rabies vaccine (C). MR = Vaccinated with multivalent and rabies vaccine. M = Vaccinated with multivalent vaccine. R = Vaccinated with rabies vaccine. C = Not vaccinated.

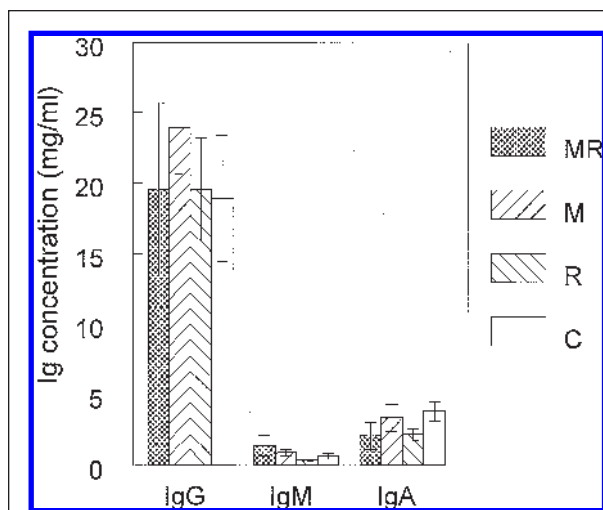


Figure 2—Mean ($\pm$ SEM) concentration of IgG, IgM, and IgA in the serum of dogs after vaccination at 3 years of age. See Figure 1 for key.

tration of BFN-specific IgE, but not the BSA-specific IgE, was significantly correlated with the rabies vaccine-specific IgE ($r = 0.776$, $P < 0.001$). There was no

significant correlation between BSA- and BFN-specific IgE ($r = 0.362$, $P = 0.117$).

To determine whether the serum IgE concentration remained high following vaccination, blood samples were collected before and at 2 weeks after vaccination at 4 years of age and then monthly for 3 months. Serum total IgE concentration was increased in 6 of the 10 dogs that were vaccinated for rabies (MR- and R-group dogs). The increase was transient

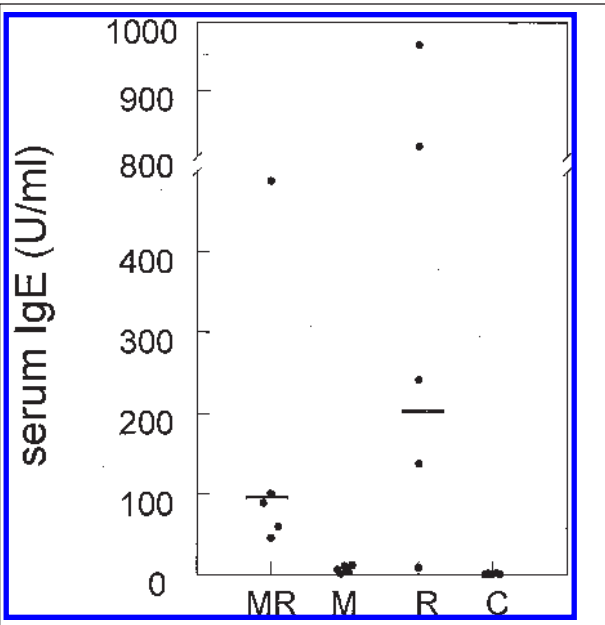


Figure 3—Concentration of IgE in the serum of dogs at 14 days after vaccination at 3 years of age. Each dot represents a dog. Horizontal bars in groups MR and R indicate the geometric mean. See Figure 1 for key.

as it peaked at 2 weeks and returned to baseline values in all but 2 dogs by 6 weeks following vaccination (Fig 5). The BSA- and BFN-specific IgE concentration increased in 8 and 5 dogs that were vaccinated for rabies, respectively. The concentration of BSA-specific IgE remained high 14 weeks following vaccination in 5 dogs. The concentration of BFN-specific IgE was still increased at 14 weeks after vaccination in 2 dogs.

The concentration of aluminum in the rabies vaccine was 32.6 ppm and in the multivalent vaccine 0.06 ppm, as determined by ICP spectrometry. By comparison, the aluminum content of tissue culture medium (RPMI-1640 with 10% fetal calf serum) was 0.12 ppm.

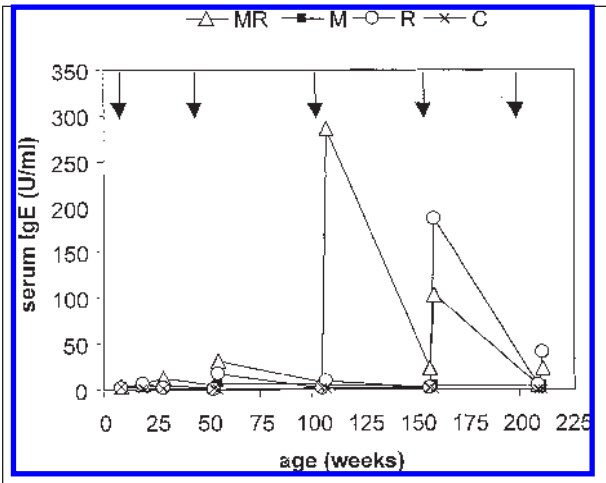


Figure 4—Concentration of serum IgE (geometric mean) in the 4 groups of dogs. Arrows indicate the time of vaccination for rabies. See Figure 1 for key.

Table 1—Serum IgE and IgG reactivity of dogs with vaccine components 2 weeks after vaccination at 3 years of age with a multivalent and rabies vaccine (MR-group dogs, $n = 5$), multivalent vaccine only (M-group dogs, 5), rabies vaccine only (R-group dogs, 5), or 0.9% NaCl only (C-group dogs, 5)

Dog group and number	IgE (OD_{405})			IgG (OD_{405})			
	Rabies vaccine	V5	Diluent	BSA	BFN	BSA	BFN
MR 1	0.632	< 0.1	0.16	< 0.1	< 0.1	2.680	2.806
MR 2	0.131	< 0.1	0.203	0.151	< 0.1	2.717	3.080
MR 3	1.559	< 0.1	0.45	0.343	0.751	2.842	2.818
MR 4	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	2.876	2.880
MR 5	0.745	< 0.1	< 0.1	< 0.1	0.101	2.549	2.795
M 1	0.102	< 0.1	0.149	< 0.1	< 0.1	2.740	3.093
M 2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	3.166	2.633
M 3	0.106	< 0.1	< 0.1	< 0.1	< 0.1	3.078	1.407
M 4	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	2.912	0.750
M 5	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	3.002	2.873
R 1	0.179	< 0.1	0.325	0.931	< 0.1	3.135	2.516
R 2	0.365	< 0.1	0.377	0.345	0.611	2.623	3.106
R 3	0.168	< 0.1	0.110	0.282	< 0.1	2.551	2.553
R 4	0.123	< 0.1	0.175	< 0.1	< 0.1	2.596	3.128
R 5	0.130	< 0.1	< 0.1	0.257	< 0.1	2.650	2.785
C 1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C 2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.103	< 0.1
C 3	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C 4	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C 5	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

V5 = Lyophilized virus component of the multivalent vaccine. BSA = Bovine serum albumin. BFN = Bovine fibronectin. OD_{405} = Optical density read at 405 nm.

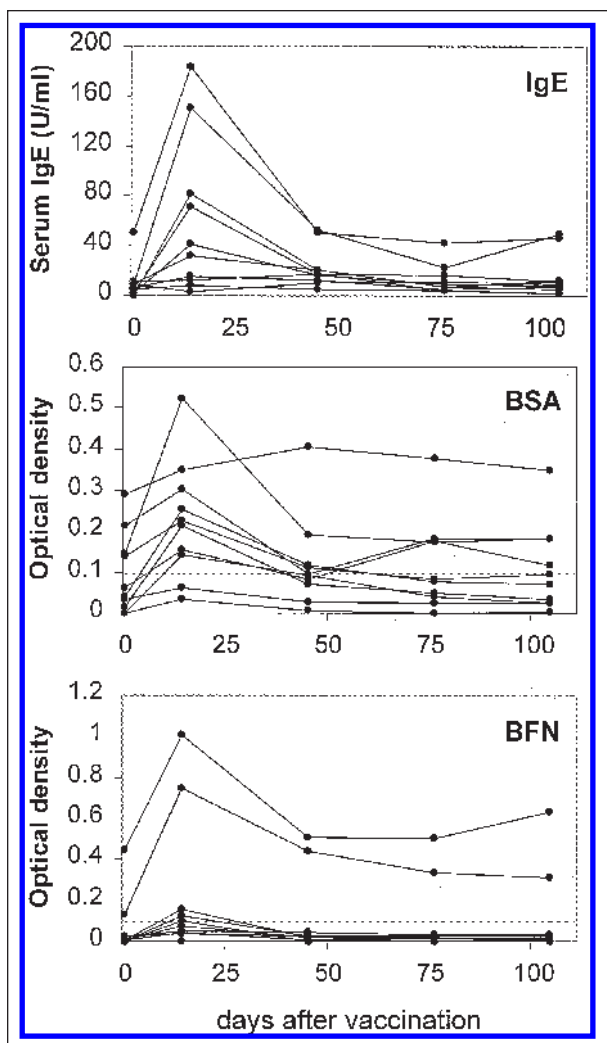


Figure 5—Changes in serum total IgE concentrations and bovine serum albumin (BSA)- and bovine fibronectin (BFN)-specific IgE of individual dogs following vaccination for rabies at 4 years of age. Each line represents 1 dog. IgE antibodies against BSA and BFN at an optical density < 0.1 (indicated by the dotted line) were not considered important.

Discussion

Results of our study indicate that vaccination of dogs with an inactivated rabies vaccine caused a significant but transient increase in the serum total IgE concentration in dogs. Unvaccinated dogs, raised and housed in a clean indoor environment, had a low serum IgE concentration. In contrast to the rabies vaccine, vaccination with a multivalent vaccine only slightly increased the serum IgE concentration. The effect of the rabies vaccine on the IgE concentration was most likely caused by the aluminum adjuvant in the vaccine, because aluminum salts have been shown to promote the induction of antigen-specific IgE antibodies in dogs^{23,24} as well as rodents²⁵ and human beings.^{6,7} A similar effect was not seen with the multivalent vaccine that did not contain an aluminum adjuvant as verified by ICP spectrometry. However, we cannot exclude the possibility that components other than the aluminum adjuvant contributed to the IgE-inducing effect of the rabies vaccine.

The increase in serum total IgE concentration was associated with the formation of IgE antibodies against vaccine components, including BSA and BFN, in most dogs. The BSA and BFN are presumably present in vaccines as residues from the tissue culture used to generate the vaccine viruses, and BSA may also be added to vaccines as a stabilizer.¹⁷⁻²⁰ Similarly, studies^{8,26} in humans have documented IgE antibodies against gelatin, which is used as a stabilizer in some vaccines.

There was no correlation between the concentration of antigen-specific and total IgE, and BSA- and BFN-specific IgE persisted, whereas serum total IgE concentration rapidly returned to baseline concentrations in most dogs. A similar discordance has been observed in humans following immunization with aluminum-adsorbed tetanus toxoid⁶ and in rats following injection of aluminum-adsorbed ovalbumin.²⁷ This suggests that vaccine-specific IgE contributes little to the overall increase in serum total IgE concentration and that total IgE is differently regulated from antigen-specific IgE.

There was distinct variation in the serum total IgE concentration and antigen-specific IgE among individual dogs consistent with a strong genetic influence on IgE production in dogs.⁹ In this small study population, there was no association of high or low IgE responders with a particular litter of origin (data not shown). Several genetic loci have been identified that control the serum IgE concentration in humans.^{28,29} Results of our study suggest that vaccination for rabies can be used to identify genes that control the increase serum concentrations of antigen-specific and total IgE on antigenic stimulation in the outbred dog population.

Serum total IgE concentration rapidly declined after the initial increase following vaccination, but antigen-specific IgE persisted for months in several dogs. Yet, none of the Beagles with high serum concentrations of antigen-specific IgE developed evidence of allergic disease or an acute allergic reaction after revaccination. A possible explanation is that the vaccines contain only small amounts of antigens, most of which may be initially adsorbed to the aluminum adjuvant. The small quantities of antigens that are released over time may not be sufficient to cause significant degranulation of sensitized mast cells and basophils. This is supported by a study³⁰ in children in which local reactions to a plain vaccine were more severe than to an aluminum-adsorbed vaccine. Similarly, aluminum hydroxide adjuvants can prevent adverse reactions to lipopolysaccharides by adsorption to the aluminum particles.^{31,32} An alternative explanation for the lack of allergic reactions is the existence of different isoforms of IgE. Differential RNA splicing of the epsilon RNA-transcript results in various mRNA isoforms that are translated in IgE molecules that may differ in glycosylation and function.^{33,34} Evidence for functional heterogeneity of IgE comes from studies^{35,36} of histamine-releasing factor. This cytokine induces the release of histamine from basophils in the presence of IgE from some, but not all donors.

Immunization of rats with ovalbumin and a pertussis adjuvant induced ovalbumin-specific IgE and prolonged hypersensitivity of the intestine following injection of ovalbumin into the intestinal lumen.³⁷ This sug-

gests that IgE antibodies specific for bovine proteins that were induced by the vaccination of dogs in our study for rabies could cause intestinal hypersensitivity on feeding rations containing bovine proteins. Such hypersensitivity could result in clinical food allergy in susceptible dogs. Hypersensitivity to beef products is common in dogs with food allergy.^{38,39} Indeed, subcutaneous immunization of dogs with bovine proteins (beef extracts and dried cow milk) mixed with aluminum adjuvant has been used to develop a model of food allergy in genetically predisposed dogs.¹⁰ Allergic reactions to bovine proteins in cow's milk are not uncommon in infants and young children.⁴⁰ Whether human vaccines contain bovine tissue culture components that can induce IgE antibodies remains to be investigated, but the role of vaccination in the development of certain forms of food allergies merits further studies.

In summary, vaccination for rabies caused a significant but transient increase in serum total IgE concentration in dogs. Vaccination history should be considered in the interpretation of serum total IgE concentration. Some of the IgE was specific for bovine proteins that were presumably present in vaccines as tissue culture residues.

^aVanguard 5/L, Pfizer, Groton, Conn.

^bImrab-3, Merial, Athens, Ga.

^cCornell Diagnostic Laboratory, Cornell University, Ithaca, NY.

^dDepartment of Veterinary Diagnosis, KSU Veterinary Medical Center, Kan.

^eBethyl Laboratories, Montgomery, Tex.

^fMolecular Devices, Menlo Park, Calif.

^gSouthern Biotechnology, Birmingham, Ala.

^hPlasma 400 ICP spectrometer, Perkin Elmer, Boston, Mass.

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