

Original article

Allergen immunotherapy with heat-killed *Listeria monocytogenes* alleviates peanut and food-induced anaphylaxis in dogs

Background: Heat-killed *Listeria monocytogenes* (HKL) potently stimulates interferon (IFN)- γ production in CD4 T-lymphocytes, and when used as adjuvant for immunotherapy, reduces immunoglobulin (Ig)E production and reverses established allergen-induced airway hyperreactivity (AHR) in a murine model of asthma. We asked if such treatment could decrease established peanut-induced anaphylaxis or cow's milk-induced food allergy in highly food-allergic dogs.

Methods: We therefore studied four 4-year-old atopic colony dogs extremely allergic to peanut (Group I), as well as five 7-year-old dogs very allergic to wheat, milk and other foods (Group II). All dogs experienced marked allergic symptoms, including vomiting and diarrhea on oral challenge with the relevant foods. The dogs were then vaccinated once subcutaneously with peanut or milk and wheat with HKL emulsified in incomplete Freund's adjuvant.

Results: Following vaccination of the allergic dogs with HKL and allergen, oral challenges with peanut (Group I) or milk (Group II) elicited only minor or no symptoms. In addition, skin test end-point titrations showed marked reductions for > 10 weeks after treatment, and levels of Ara h 1-specific IgE in serum of peanut sensitive dogs, as demonstrated by immunoblotting, were greatly reduced by treatment with HKL plus peanut allergen.

Conclusions: Thus, HKL plus allergen treatment markedly improved established food allergic responses in dogs, suggesting that such an immunotherapy strategy in humans might greatly improve individuals with food allergy and anaphylaxis.

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Peanut and tree nut allergy is a significant public health problem that can result in life threatening anaphylaxis. Peanut or tree nut allergy affects approximately 1.5 million people in the USA (1), and accidental ingestion of an implicated food by allergic individuals is estimated to cause about 150 deaths/year (2). In addition, allergy to all foods affects 6–8% of children under age 4 and about 2% of the general population. Because there are no practical and successful immunotherapies for food allergies, the major focus of treatment is avoidance of offending foods. However, inadvertent exposure to food allergens and the resultant serious or fatal reactions remain a grave risk for food sensitive individuals.

Alternative approaches for treatment of patients with food allergy are being studied intensely, with the goal of developing immunotherapies that might reverse or cure food sensitivity (3). Immunotherapies utilizing adjuvants such as *Listeria monocytogenes*, a Gram-positive bacterium that causes Listeriosis and potently activates T cells, have been shown to reverse Th2 biased immune responses and

airway hyperresponsiveness in the lungs of mice. Heat-killed *Listeria* (HKL) mixed with allergen (ovalbumin, OVA) in incomplete Freund's adjuvant (IFA) markedly abrogated established OVA-specific Th2 responses, airway hyperreactivity (AHR) and airway inflammation (4, 5). There was a significant decrease in allergen-specific immunoglobulin (Ig)E and interleukin (IL)-4 and a concurrent increase in allergen-specific IgG2a and interferon (IFN)- γ produced by lymph node cells. The HKL-effect reduced airway eosinophilia and mucus production and was associated with the production of IL-18. In addition, neutralization of IL-12 with monoclonal anti-IL-12 at the time of HKL-OVA-IFA treatment blocked the enhancement of IFN- γ and the reduction in IL-4, indicating that IL-12 was also responsible for the effect. Thus HKL-therapy was highly effective for the treatment of an ongoing Th-2 type immune response, and converted a pathogenic response into a protective response.

We asked whether the conversion of a Th2 allergic response into a protective response by HKL could be

demonstrated in a model of food allergy in nonmurine species. Food allergy has been particularly difficult to treat in humans, and immunotherapy for food allergy has been associated with unacceptable rates of systemic reactions (6). To establish a system that might reliably reflect human disease, we used a large animal model with inbred food-allergic dogs (7) rather than small rodents. We showed that immunotherapy with HKL as adjuvant could diminish established food allergy in dogs, suggesting that such adjuvant therapy might be effective in the treatment of food allergy in humans.

Methods

Animals

Inbred atopic dogs were used and housed at the Center of Laboratory Animal Science, University of California, Davis, Veterinary School in AAAALAC-associated facilities and were cared for according to Institute of Laboratory Animal Resources Guidelines (8). At birth, four dogs were immunized subcutaneously with 1 µg peanut extract (Miles Inc, West Haven, CT) 1 : 10 w/v in 200 ml saline and 200 mg alum along with four other allergens (cow's milk, soy, barley and ragweed) (Group I). Furthermore, five additional dogs were immunized subcutaneously at birth with cow's milk, soy, beef, wheat, and ragweed, each in identical doses (Group II) (7). Protein content was measured by using the BioRad protein assay kit (BioRad Inc., Hercules, CA). At 3 weeks of age, the pups were immunized subcutaneously in the shoulder with 1.0 ml live attenuated canine distemper vaccine (Schering-Plough Animal Health, Omaha, NB) and at 7 and 11 weeks of age, with attenuated live distemper-adenovirus type 2, canine parainfluenza-parvovirus vaccine (Fort Dodge Laboratories, Fort Dodge, IO). Two and 9 days after each vaccination, the dogs were injected with the same allergens as on day 1. Booster immunizations of 10 µg allergens in alum were given bimonthly in the axilla to maintain the food allergic state.

The dogs produced IgE antibodies to the immunized foods by 3 months of age. The responses to peanut were especially strong, as measured by dot blots and direct skin test titrations. After 2.5 years of age, upon oral challenge with 5–20 g ground peanut, the dogs consistently experienced vomiting and lethargy.

Group I. Peanut-allergen treated dogs

Four of the dogs participated in a study comparing allergic responses to peanut, walnut and Brazil nut (9). At 4 years of age, these food allergic dogs (7FB4, 5, 6, and 9), Group I, were immunized with 1.0 ml HKL [10^9 organisms in phosphate buffered saline (PBS)] mixed with peanut antigen, 1.0 ml of peanut extract 1 : 10w/v (Hollister Stier, Spokane, WA), emulsified in 2.0 ml of IFA (Difco Laboratories, Detroit, MI) (4 ml/dog), injected subcutaneously into the axilla (approximately 10 mg of allergen/dog). One dog, 7FB 9, received a second injection 4 weeks later.

Group II. Immunization with HKL-wheat or cow's milk

At 7 years of age, five other food allergic dogs were placed in Group II to study the effects of HKL for treatment of wheat and milk allergy. Three of these dogs were immunized with 1.0 ml of wheat and cow's milk extracts 1 : 10 w/v (Hollister-Stier, Spokane, WA), mixed with 1 ml PBS containing 10^9 organisms of HKL and

emulsified in 2.0 ml IFA. Four milliliters of the emulsion was injected subcutaneously into the axilla of the three dogs (6GCB-2, -3, -8) (approximately 5 mg each of wheat and milk/dog). Two dogs served as controls (6GCB-1, -7) and were injected with 2 ml IFA mixed with 2 ml PBS.

Heat-killed *Listeria* preparation

The HKL was prepared as described by Yeung et al. (4). Briefly, bacterial *Listeria monocytogenes* cultures in log-phased growth were harvested, centrifuged and washed 3× in PBS. The recovered bacteria were resuspended in PBS and incubated at 80°C for 1 h. After two additional washes in PBS, the absence of viable colonies were confirmed by lack of growth in nutrient agar plates. Bacterial concentration was enumerated by comparing absorbance of a serial dilution of HKL at 570 nm with a standard dilution of known concentration of *Listeria* that had been previously enumerated by counting growth of colonies from serial dilutions of bacteria placed on nutrient agar. HKL was kept at –80°C.

Skin test titration

Serial 10-fold dilutions of 1 : 10 w/v food allergen extracts (peanut, wheat, cow's milk, beef, ragweed) (Miles Inc, West Haven, CT) were injected intradermally on the shaved ventral abdomen of the dogs 5 min after intravenous injection of 0.5% Evans Blue dye (0.2 ml/kg) (Sigma Chemical Co, St Louis, MO), which help to better visualize wheal skin reactions. The end-point of titration was the maximum dilution that gave a 5 × 5 mm blue wheal (Fig. 1).

Skin test titrations were performed twice, 4 weeks apart, before treatment with HKL + peanut in Group I and HKL plus wheat and cow's milk in Group II. After vaccination with the HKL-allergen, skin test titrations were performed at 1, 2, 6, 8 and 10 weeks for peanut, milk and ragweed in Group I, and at 1, 3, and 13 weeks for wheat, cow's milk, beef and ragweed in Group II.

Oral food challenges

Dogs were monitored individually in their kennels for 3 days prior to food challenges to ensure normal appetite and the absence of diarrhea and vomiting (7, 9). Stools were graded as either firm,



Figure 1. Skin test end-point titration with cow's milk in control dog, GCB-1.

semi-soft or runny/loose consistency. On the day of challenge, food was withheld to decrease the chance of gastric torsion. For Group I dogs, challenge with freshly ground peanut prior to vaccination was initiated at 200 mg, followed by 500 mg, 1 g, and 2 g at 30 min intervals. One dog did not react at these doses and was challenged the next week with 4 g of peanut, followed by an additional 4 g before reacting. Prior to vaccination anaphylaxis was induced in all four dogs by graded challenge of peanut with doses up to 8 g of peanut. After vaccination, the challenges doses were 200 mg, 500 mg, 1 g and 2 g the first day, followed 1 week later if needed by 4, 6 and 10 g challenges (total dose up to 20 g). Ground peanut was moistened with water just before challenge to form a slurry, which was placed on the tongue to enable swallowing. The dogs in Group II were challenged with cow's milk (0.2 g) 1 week before vaccination with HKL and at 2 and 4 months after vaccination. Dry cow's milk powder moistened with water was used.

Following oral challenge, the dogs were monitored for vomiting, swelling, naso-ocular signs, pallor of oral mucosa, lethargy and diarrhea. Direct observation continued for 3 h after challenges. The dogs were then monitored at 5 and 7–8 h, then left overnight with stool/kennel checks three times per day for the next 3 days. If a dog had a significant anaphylactic reaction requiring both epinephrine and fluid resuscitation, it was taken to the on-site clinic for treatment and observation. Symptom scores were generated by adding the number of episodes of diarrhea with the number of vomiting episodes. The number of episodes of vomiting was multiplied by a factor of 3, as vomiting was considered to be more specific than diarrhea as a symptom of anaphylaxis.

IgE immunoblotting of sera

Sera were obtained before the HKL-peanut immunization and 3 months after the treatment. Electrophoresis and transfer of reduced peanut protein extracts to 0.22 nm nitrocellulose membrane (MSI, Westborough, MA) were carried out as previously described (10). Blots were cut into strips containing about 25 µg of protein per 4 mm and then blocked for 1 h at room temperature in PBS/3% nonfat dry milk/0.2% Triton X-100 (TX-100). Diluted sera from the immunized dogs or pooled sera from control nonatopic dogs (1 : 5 in PBS/3% nonfat dry milk/0.2% TX-100) were added to strips and incubated overnight at 4°C. Additional strips were incubated with PBS/3% nonfat dry milk/0.2% TX-100 as buffer control to monitor for nonspecific binding of anti-canine IgE polyclonal antibodies. The strips were washed three times for 20 min in PBS/0.01% TX-100 and incubated overnight with anti-canine IgE-horse radish peroxidase (CMG, Fribourg, Switzerland). Strips were then washed again and developed with the TMG Peroxidase Substrate Kit (Vector Laboratories Inc, Burlingame, CA).

Image densitometry

The NIH software for Windows Scion Image Beta 4.02 for Windows OS NT 2000 (Scion Corporation, Frederick, MD) was utilized. The integrated density of the defined area of study was compared in the region of binding to Ara h 1 from immunoblots before and after HKL plus peanut vaccination.

Statistics

Significance was established using student *t*-tests of log transformed data.

Results

Vaccination greatly improves skin test end-point titrations

Effect of HKL-peanut vaccination. Group I dogs consisted of four dogs with strong sensitivity to peanuts. At baseline, immediate wheal skin test reactions were induced in the dogs with a (geometric) mean of only 0.075 µg of peanut extract (Fig. 2), indicating exquisite sensitivity to peanut. Following vaccination with mixed HKL-peanut antigen, skin test reactivity improved mark-

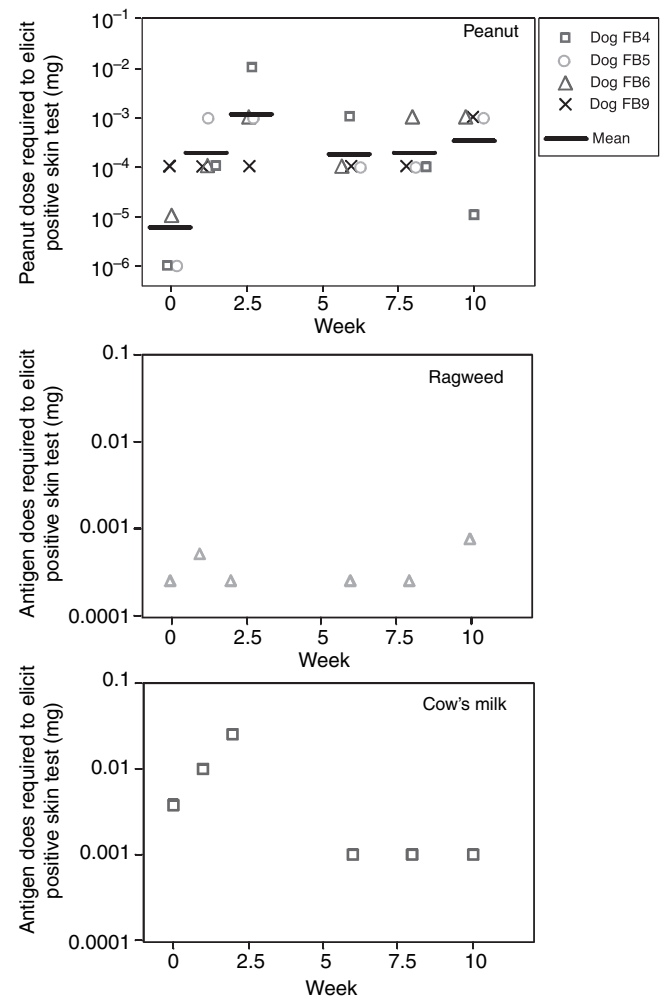


Figure 2. Treatment with peanut + heat-killed *Listeria monocytogenes* (HKL) reduces skin test reactivity to peanut but not to milk or ragweed. Geometric mean skin test end-point titrations in Group I HKL-peanut treated dogs with peanut, milk and ragweed. (A) Data represents milligrams of peanut antigen injected to elicit a positive skin test. Each symbol represents an individual dog. Horizontal bars represent the geometric mean of injected antigen. $P = 0.04$ at 10 weeks after vaccination. (B) Data represents milligrams of milk or ragweed antigen injected to elicit a positive skin test (geometric mean of injected antigen). There was no statistical difference at 10 weeks after vaccination.

edly in three of the four dogs, such that much greater amounts of peanut extract was required to elicit skin test reactivity. At 1 and 2 weeks post-treatment, the dogs required 10 and 25 μ g of peanut antigen, respectively, to elicit a wheal (133 and 300 times the original dose). At 6–10 weeks post-treatment, elicitation of skin test reactivity required 2.5 and 5 μ g peanut (33 and 66 times the original dose), suggesting that treatment with peanut plus HKL greatly reduced peanut-specific IgE levels in three of the four dogs. In all four dogs, skin test titration results were significant at week 10 with $P = 0.04$.

Vaccination effects are antigen-specific. The Group I dogs were allergic not only to peanut but also to several other antigens including cow's milk and ragweed antigens. At two time points prior to vaccination with peanut in HKL, elicitation of an immediate skin reaction to milk required 2.5 and 5 μ g of milk allergen. Two weeks after vaccination, there was a two- to 10-fold increase in milk antigen dose required to induce a wheal, but the dose of milk required to induce a wheal fell to baseline at 10 weeks (Fig. 2B). At baseline, 250 ng of ragweed antigen was required to induce a wheal skin test reaction, which increased by twofold at 1-week post-treatment and rose to threefold of baseline at 10 weeks. These results indicated that immunization with peanut mixed with HKL greatly reduced skin test reactivity to peanut in three of the four dogs, while the skin test reaction to control (nonpeanut) antigens was not affected, indicating that the effects of vaccination were antigen specific.

Effect of HKL-Wheat-Cow's milk vaccination. The second group of dogs, Group II, consisted of five dogs, which were highly allergic to wheat, cow's milk, beef and ragweed. Three of the dogs were vaccinated with HKL mixed with wheat and milk allergens. The remaining two dogs were injected with IFA alone without allergen.

At baseline the three treated dogs required 10 pg of wheat antigen to induce a wheal skin test reaction. At 1-week post-treatment, the wheat antigen dose required for a wheal reaction increased threefold, and by 3 and 13 weeks after vaccination, the dose of wheat required rose 100-fold. At baseline the dogs required 66 ng of cow's milk protein to elicit a wheal skin test reaction. By 1 week the milk allergen dose required doubled, and by 3 and 13 weeks post-treatment, the dose of cow's milk required for a wheal reaction increased 10- and 50-fold, respectively (Fig. 3). In contrast, skin test reactivity in these dogs in response to beef and ragweed antigen did not change from baseline. Furthermore, in the two control dogs immunized with PBS/IFA without HKL, there was essentially no change in the dose required for a wheal reaction to any antigen over the course of the experiment. These results indicated that improvement in immediate skin test reactivity to wheat and cow's milk following vaccination with HKL mixed with wheat and

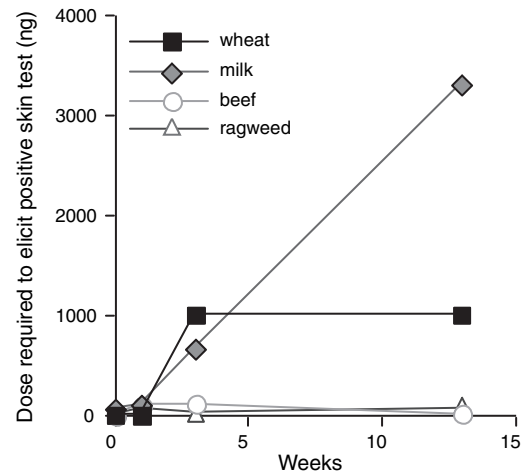


Figure 3. Mean skin test end-point titrations in Group II heat-killed *Listeria monocytogenes*-milk and wheat-treated dogs with milk, wheat, beef, and ragweed. Each point represents the geometric mean of dose required in five dogs.

cow's milk antigen was significant and antigen specific, and unlikely to be related to administration of IFA.

IgE immunoblotting. The improvement in skin test end point titrations for the dogs in Group I was confirmed with IgE immunoblotting, measuring IgE antibody against specific peanut antigens in serum samples from the four dogs taken before and after treatment with HKL plus peanut antigen. Figure 4 shows that prior to treatment the sera from all four dogs contained IgE strongly reacting with Ara h1 (66 kDa band). However, following treatment with HKL plus peanut antigen, the band intensity for Ara h1, the major peanut allergen, was greatly diminished in all four dogs. The decrease in the integral density calculation for the Ara h 1 region on the blots was similar for all dogs, 95, 98, 95, and 93% reduction for dogs 7FB4, 7FB5, 7FB6, and 7FB9, respectively. The bands for IgE against other peanut polypeptides was relatively unaffected, although the signal intensity to polypeptides in the region 21–25 kD in 7FB4 and at 31 kD in 7FB5 also appeared diminished after treatment with HKL plus peanut.

Vaccination greatly improves the response on oral food challenge

Effect of HKL-peanut vaccination. Prior to vaccination with HKL, the four dogs in Group I developed symptoms after oral challenge with a geometric mean dose of 0.63 g of peanuts (range 0.2–8 g), indicating great sensitivity to peanut. Two of the dogs reacted to the lowest challenge dose of 0.2 g of ground peanut (approximately 0.05 g of peanut protein), and all four dogs developed severe symptoms, requiring treatment with subcutaneous epinephrine and diphenhydramine. However, Fig. 5 shows that after vaccination with HKL plus peanut, the oral

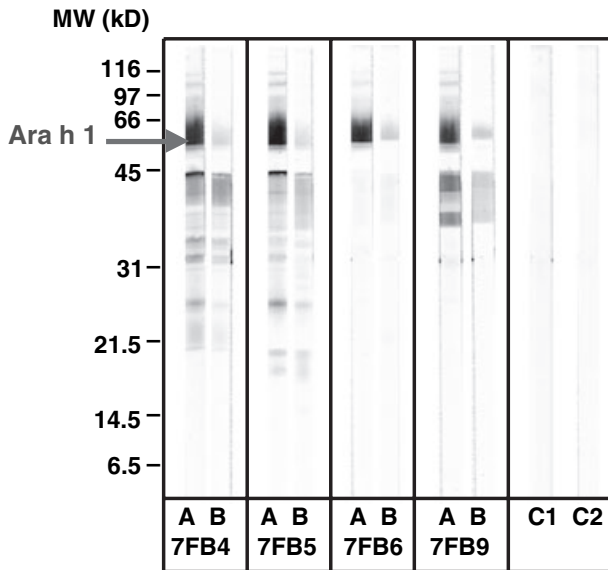


Figure 4. Immunoglobulin (Ig)E-immunoblots with sera from four individual dogs before (A) and after (B) heat-killed *Listeria monocytogenes* (HKL)-peanut treatment. C = controls. Ara h1 specific IgE was clearly reduced in all four dogs after treatment with HKL + peanut.

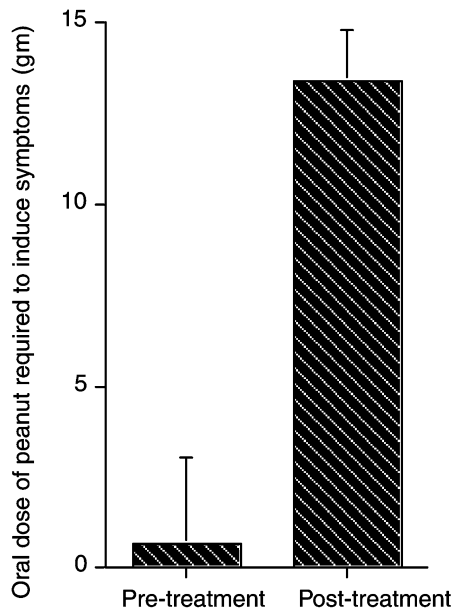


Figure 5. Geometric means of antigen dose required to elicit symptoms in Group I dogs, before and 3 months after heat-killed *Listeria monocytogenes* (HKL)-peanut treatment. The dose of peanut tolerated by the dogs greatly increased after treatment with HKL + peanut ($P = 0.03$).

challenge dose required to elicit symptoms rose greatly in all animals to a geometric mean of > 13 g, a significant increase with $P = 0.03$ (paired student t -test). In fact three

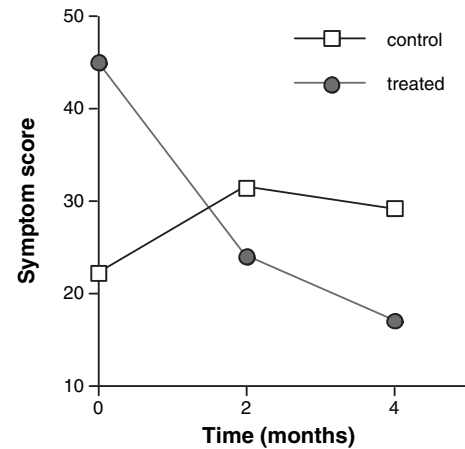


Figure 6. Symptom scores after oral feeding challenges with cow's milk in Group II before, 2 and 4 months after heat-killed *Listeria monocytogenes*-milk, wheat treatment.

of the dogs were able to tolerate the highest dose of 20 g ground peanut without developing any symptoms, including dog 7FB5, which prior to treatment had the severest reactions after challenge with 0.2 g of peanut. Therefore this dog had more than a 100-fold increase in the amount of peanut tolerated on oral challenge.

Effect of HKL-wheat-cow's milk vaccination. In Group II, the dogs were challenged with 0.2 g of cow's milk orally and then observed for symptoms. Prior to treatment all of the dogs reacted to the challenge with diarrhea (observed over 3 days), and four of the five dogs had vomiting. At 2 and 4 months postvaccination with HKL plus milk, there was a significant reduction ($\sim 60\%$) in symptoms observed in the HKL-treated dogs, while there was a slight increase ($\sim 5\%$) in the symptoms observed in the control dogs (Fig. 6). None of the HKL plus milk treated dogs developed vomiting after oral milk challenge at 2 and 4 months post-treatment. In addition, the HKL treated dogs had a 50% reduction in the number of stools observed over 3 days (12–5.6 stools), while the control treated dogs had an increase in the number of stools observed (9.5–13 stools).

Discussion

In this study, we demonstrated that vaccination of highly food allergic dogs with HKL plus food allergens (peanut or cow's milk) in one or two doses greatly diminished or abolished allergic symptoms, including vomiting and diarrhea, that developed following oral challenge with the allergen. The clinical benefit observed with oral food challenge was associated with marked falls in end-point titration immediate skin tests to the relevant food, and near disappearance of IgE antibodies to Ara h 1 in peanut

allergic dogs. In wheat treated dogs, a decrease in skin test sensitivity to wheat was also observed. These results demonstrate that vaccination with HKL plus food allergens is highly effective in the treatment of well-established, long-standing (4+ years) food allergy in dogs.

We studied the effects of vaccination of food extract with HKL as an adjuvant in dogs, because other animal models of food allergy may not closely reflect food allergy in humans. In mouse models of food allergy, sensitization often requires the use of mice deficient in TLR4 (C3H/HeJ), intragastric challenge with allergen, and observation for symptoms such as scratching and rubbing around snout and head, reduced activity and drops in body temperature (11, 12). In contrast, in our model, dogs, which are evolutionarily more complex than mice and closer to humans, were challenged orally with clinically important allergens (peanut and cow's milk), and were observed for vomiting and diarrhea, which are symptoms commonly observed in humans with food reactions. Thus the dog model of food allergy replicates many of the characteristics in humans of food-induced anaphylaxis, and therefore is a valuable model with which to study therapies for human food allergy and anaphylaxis.

In our dog model of food allergy, we demonstrated that immunization with just one dose of allergen plus HKL as an adjuvant greatly reduced immediate skin test sensitivity and greatly reduced food allergy induced symptoms in an antigen specific fashion in dogs with long standing hypersensitivity (up to 7 years). This is similar to the rapid improvement in Th2 driven responses using HKL as an adjuvant for immunotherapy in a mouse model of asthma (4). Moreover, the effect of a single dose of HKL + food allergen in dogs lasted for at least 5 months, suggesting that HKL has persistent long term antigen-specific effects on the immune system, presumably because vaccination with HKL alters the underlying immunological basis of allergy. In contrast, passive therapies for food allergy, for example with administration of anti-IgE monoclonal antibody (mAb), have only transient effects. Thus treatment of patients with peanut allergy with an anti-IgE mAb increased the dose of peanut tolerated on oral challenge by patients (13), however, the protective effect of anti-IgE mAb therapy rapidly wanes once the drug is discontinued.

Treatment with HKL as adjuvant is very effective because it rapidly activates innate immunity and induces potent adaptive immunity and antigen-specific protective immune responses that reduces allergen specific Th2 responses. Immunologically, *Listeria* activates the innate immune system by activating APCs (dendritic cells and monocyte/macrophages) and NK cells. *Listeria* express several pathogen associated molecular patterns (PAMPs) that are recognized by specific receptors (14), including lipoproteins (lipoteichoic acid) that bind to TLR2, flagellin that bind to TLR5 (15) and CpG motifs in its DNA that bind to TLR9 (16). The effect of HKL is clearly not restricted to the activation of TLR9, because the beneficial

adjuvant effects of HKL are still observed in TLR9^{-/-} mice (D. T. Umetsu, unpublished data). Nevertheless, TLR9 activation by CpG DNA rapidly induces high levels of IL-12 and IL-18 (17) by APC, which then causes NK cells to produce large quantities of IFN- γ . In addition, *Listeria* induces TNF- α and nitrous oxide production in macrophages, which help to clear the bacteria (18). The adaptor molecule MyD88 (myeloid Differentiation factor 88) (18) is critical for signaling by all TLRs including TLR9, and in MyD88^{-/-} mice TLR activation is abolished, resulting in marked reduction in IFN- γ , TNF- α and NO production, and high susceptibility to *Listeria* (19). Because activation of specific combinations of TLRs result in the development of specific forms of immunity, the combination of TLRs activated by HKL may be particularly effective in inducing immunity that protects against allergy and asthma.

Activation of the innate immunity by HKL has major effects on adaptive immunity. For example, treatment with HKL plus peanut was associated with disappearance of IgE directed against Ara h1, the major peanut allergen in humans, and presumably the dominant allergen in canine anaphylaxis. Reduction in IgE synthesis by HKL as adjuvant occurs as a result of IL-12 and IFN- γ production (4, 5), which also greatly augments CD4 Th1 responses. While Th1 responses have been shown to inhibit Th2 responses particularly in infectious disease models (20, 21), HKL also induces large quantities of IL-10, and the combination of IFN- γ and IL-10 may be particularly effective in limiting strong Th2 responses. Moreover, the HKL protective effect in allergy is blocked by treatment with anti-IL-10 mAb and with anti-TGF- β mAb, suggesting that allergen-specific tolerance mechanisms may be induced with HKL plus allergen. For example, regulatory T cells, which produce IL-10 or TGF- β (22), and which are associated with both oral and respiratory tolerance, have been shown to have potent anti-inflammatory effects in respiratory and intestinal mucosa, and may be induced with HKL as adjuvant (23). The induction of regulatory T cells as well as Th1 responses, may explain the potent anti-inflammatory effects of HKL as an adjuvant.

Several other studies have examined allergen immunotherapy for the treatment of food allergy. For example, in a mouse model of peanut induced anaphylaxis, treatment with plasmids containing the cDNA for Ara h 2 complexed to chitosan particles administered orally, was effective in preventing Ara h 2 sensitization and subsequent anaphylaxis (12). The beneficial effect in this preventive gene delivery vaccine model is likely to be due in part to the presence of CpG motifs in the plasmid DNA, which utilize TLR9 innate immune pathways to block the development of Th2 responses. However, gene therapy with allergens, either complexed to chitosan or administered as naked DNA appears to be ineffective in reversing established allergic disease (24), although treatment with CpG conjugated to allergen proteins appears to be effective in reversing established allergic disease in humans (25). In

any case, treatment with chitosan-allergen DNA plasmids would be of limited use clinically if it could not clearly reverse established allergic disease. In contrast, allergen immunotherapy with HKL as adjuvant reversed established disease in a mouse model of food allergy (3), as it did in our dog model of food allergy and in our mouse models of asthma (4). In the mouse model of food allergy, HKL administered with recombinant Ara h 1, 2 and 3 prevented the development of peanut induced anaphylaxis in peanut sensitized mice, as measured by prevention of reductions in core body temperature and peak expiratory flow using whole body plethysmography. In our study, we examined symptoms of food allergy reactions (vomiting and diarrhea), which are commonly seen in human food allergy, in a large animal model that might more reliably reflect human disease.

In summary, we demonstrated that vaccination of dog with HKL plus food allergens greatly suppressed allergic gastrointestinal and anaphylactic responses in peanut and milk-sensitive dogs. The beneficial effect was associated

with profound improvements in immediate skin test reactivity and in peanut allergic dogs, with loss of Ara h 1 specific IgE. These results indicate that HKL, which has been shown to be effective as an adjuvant in small rodents, is also effective as adjuvant in combination with specific allergen in a larger, evolutionarily more complex animal, the dog. In addition, these studies suggest that HKL as an adjuvant might be very effective as curative immunotherapy for allergic diseases, including allergic asthma and food allergy in humans.

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