

Research Note

Relative Immunogenicity of Commonly Allergenic Foods versus Rarely Allergenic and Nonallergenic Foods in Mice

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ABSTRACT

Food allergies affect 6 to 8% of children and 2% of adults in the United States. For reasons that are not clear, eight types of food account for a vast majority (~90%) of food-induced hypersensitivity reactions. In this study, C57Bl/6 mice were used to test the hypothesis that commonly allergenic foods are intrinsically more immunogenic than rarely allergenic or nonallergenic foods in allergy-susceptible hosts. Groups of mice ($n = 4$ to 5) were injected intraperitoneally with the protein extracts (plus alum as an adjuvant) from chicken eggs, peanuts, almonds, filberts-hazelnuts, walnuts, soybeans, and wheat (commonly allergenic foods) and coffee, sweet potatoes, carrots, white potatoes, cherries, lettuce, and spinach (rarely allergenic and nonallergenic foods). Primary and secondary immune responses (as measured by specific IgG1 antibody serum levels) were measured by an enzyme-linked immunosorbent assay. Proteins from peanuts, almonds, filberts, sweet potatoes, cherries, and spinach elicited robust primary and/or secondary immune responses. Proteins from eggs, walnuts, and lettuce elicited poor primary responses but significant secondary responses. In contrast, wheat, soybeans, coffee, carrots, and white potatoes elicited barely detectable to poor primary and secondary immune responses. The order of the immunogenicity levels of these foods in mice is as follows: almonds = filberts > spinach (Rubisco) > peanuts $\geq$ sweet potatoes > cherries > lettuce > walnuts > chicken eggs > carrots $\geq$ white potatoes > wheat = coffee = soybeans. In summary, these data demonstrate for the first time that: (i) foods vary widely with regard to their relative immunogenicity in allergy-susceptible hosts and (ii) intrinsic immunogenicity in mice does not distinguish commonly allergenic foods from rarely allergenic or nonallergenic foods.

Immediate hypersensitivities to food, commonly called food allergies, affect 6 to 8% of children and 2% of adults in Westernized countries (15–18). The incidence of these diseases is believed to be increasing, along with asthma and other allergic diseases, for reasons that are not clear (14). Allergic diseases typically start in infancy and tend to be chronic, causing significant morbidity and a negative impact on the economy (12). Food-induced systemic anaphylaxis has been one of the leading causes of food-associated fatalities and emergency room visits (resulting in ~29,000 visits per year in the United States alone) (15, 16, 19).

While more than 175 different food types have been documented to trigger allergic reactions in susceptible humans, 90% of food allergies are caused by eight major food types—eggs, milk, wheat, soybeans, peanuts, tree nuts, fish, and shellfish (15, 16). Furthermore, peanuts and tree nuts are the leading cause of systemic anaphylaxis and death (19). The reason why only a few food types account for the vast majority of immune-mediated adverse reactions remains unknown.

In this study, we tested the hypothesis that commonly allergenic foods are intrinsically more immunogenic than rarely allergenic or nonallergenic foods in an allergy-sus-

ceptible mouse strain. Our results suggest that although peanuts and tree nuts are more immunogenic than other commonly allergenic food types, relative immunogenicity per se does not distinguish commonly allergenic foods from rarely allergenic or nonallergenic foods.

MATERIALS AND METHODS

For this study, food extracts were purchased from Greer Labs (Lenoir, N.C.), and spinach (Rubisco) was purchased from Sigma (St. Louis, Mo.). Each food extract was tested for protein content by Lowry's method. Biotin-conjugated rat anti-mouse IgG1 antibody was purchased from BD PharMingen (San Diego, Calif.), *p*-nitro phenyl phosphate was purchased from Sigma, and streptavidin alkaline phosphatase was purchased from Jackson ImmunoResearch (West Grove, Pa.).

Mice immunization and bleeding. All mice were purchased from the Jackson Lab (Bar Harbor, Maine). Only adult animals (6 to 7 weeks of age) were used in this study. All animal procedures used were in accordance with Michigan State University policies. A group of adult C57Bl/6 mice ($n = 5$) were intraperitoneally injected with 100 μ g each of protein extracts from peanuts, almonds, filberts-hazelnuts, walnuts, and chicken eggs, plus 2.5 mg of alum as an adjuvant. Another group ($n = 5$) received 100 μ g each of protein extracts from eggs, wheat, soybeans, coffee, and sweet potatoes, plus 2.5 mg of alum as an adjuvant. A third group of mice ($n = 4$) received 100 μ g each of protein extracts from carrots, white potatoes, cherries, lettuce, and spinach (Rubisco)

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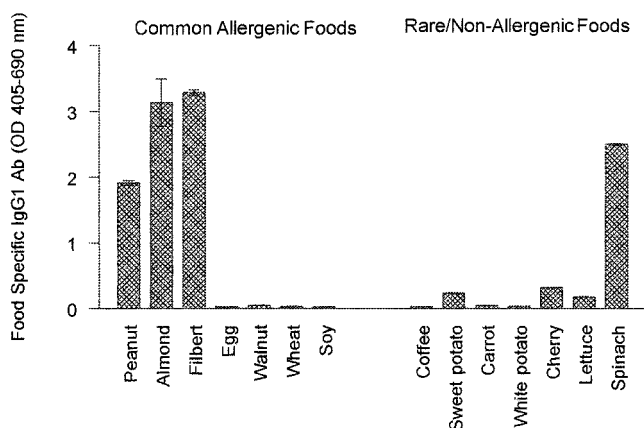


FIGURE 1. Primary immune responses to commonly allergenic foods and to rarely allergenic or nonallergenic foods in C57Bl/6 mice. Groups of mice ($n = 4$ to 5) were injected with various food extracts plus alum, and food-specific IgG1 antibody responses were evaluated 11 days after the injection. Values shown are averages $\pm$ standard errors after the background activity has been subtracted (optical density at 405 – 690 nm).

plus alum as an adjuvant. Control mice ($n = 3$) received sterile saline injections. All mice were bled 11 days after the first injection. One week later, animals received booster injections and were bled 7, 15, 32, and 62 days after the booster injection.

Food-specific IgG1 antibody measurement. An enzyme-linked immunosorbent assay (ELISA) was optimized for each of the food extracts, taking into account the background activity. All food extracts were used in the ELISA at the same concentration. The ELISA procedure was essentially the one previously described by us with modifications (20). Briefly, ELISA plates were coated with food extracts diluted in carbonate buffer (0.05 M, pH 9.6) and incubated at 4°C overnight. Unbound extract was discarded and the plates were blocked with 0.17% bovine serum albumin (BSA)–phosphate-buffered saline (PBS) at 37°C. After washing with 0.05% Tween 20 in PBS, serum samples were diluted (in 0.085% BSA, 0.05% Tween 20 in PBS) to 1:1,000 and added. Following incubation, plates were washed and a biotin-labeled anti-mouse IgG1 antibody was added. After incubation, plates were washed and streptavidin alkaline phosphatase conjugate was added. Subsequently, plates were washed and *p*-nitro phenyl phosphate substrate was added. Reactions were allowed to develop at room temperature in the dark, and plates were read for absorbance at 405 – 690 nm in an ELISA reader. All plates included negative controls (no sample background and no coating control) and a positive internal control. All samples were analyzed a minimum of two to three times.

Statistical analysis. Results of the comparison of antibody responses between food types were evaluated for significance by Student's *t* test with the SlideWrtie software program (Advanced Graphics Software Inc., Encinitas, Calif.). The statistical significance level was 0.05.

RESULTS AND DISCUSSION

Mice's serum levels of food-specific IgG1 antibody were determined during the primary response 11 days after the injection. It can be seen in Figure 1 that almonds, filberts-hazelnuts, spinach, and peanuts elicited robust antibody responses. In contrast, whereas cherries, lettuce, and

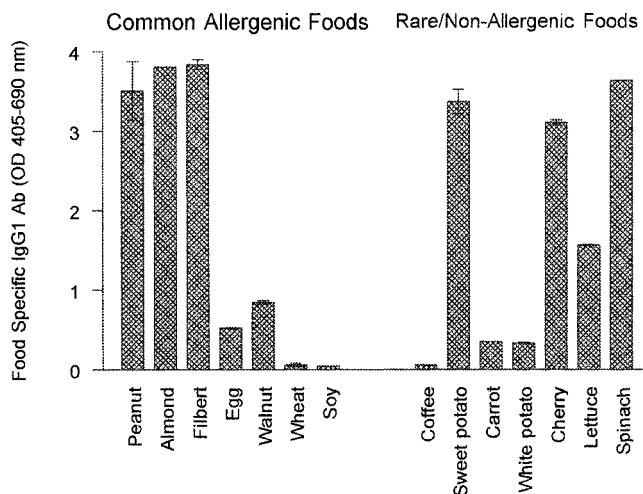


FIGURE 2. Secondary immune responses to commonly allergenic foods and to rarely allergenic or nonallergenic foods in C57Bl/6 mice. Groups of mice ($n = 4$ to 5) were injected with various food extracts plus alum, and food-specific IgG1 antibody responses were evaluated 15 days after the second injection. Values shown are averages $\pm$ standard errors after the background activity has been subtracted (optical density at 405 – 690 nm).

sweet potatoes elicited detectable antibody responses, the other foods elicited barely detectable immune responses. Thus, almonds, filberts-hazelnuts, and peanuts elicited comparable antibody responses ($P > 0.05$) that were significantly stronger than those for the other allergenic foods (eggs, walnuts, wheat, and soybeans; $P < 0.05$ for all comparisons). Sweet potatoes, cherries, lettuce, and spinach elicited significantly stronger antibody responses than did the other rarely allergenic or nonallergenic foods ($P < 0.05$).

Secondary antibody responses were evaluated on days 7, 15, 32, and 62. Since a consistent trend was evident from day 7 to day 32, a data analysis for day 15 is presented. It can be seen in Figure 2 that mice exhibited robust and comparable secondary antibody responses to almonds, filberts-hazelnuts, peanuts, sweet potatoes, cherries, and spinach ($P > 0.05$). Antibody responses to walnuts and chicken eggs were weaker than the responses to other nuts ($P < 0.05$) but stronger than the responses to wheat and soybeans ($P < 0.05$). In contrast, whereas lettuce elicited a significant antibody response, wheat, soybeans, coffee, carrots, and white potatoes elicited poor to undetectable IgG1 antibody responses. The order of the immunogenicity levels of these foods was as follows: almonds = filberts > spinach > peanuts $\geq$ sweet potatoes > cherries > lettuce > walnuts > chicken eggs > carrots $\geq$ white potatoes > wheat = coffee = soybeans.

This study produced four important novel findings: (i) food types vary widely with regard to their relative immunogenicity levels in mice, (ii) strong immunogenicity is not a feature shared by commonly allergenic food types, (iii) weak immunogenicity is not a feature shared by rarely allergenic or nonallergenic food types, and therefore (iv) immunogenicity per se does not distinguish commonly al-

lergenic foods from rarely allergenic or nonallergenic foods in the animal model used here.

Several factors influence protein immunogenicity *in vivo*, including the dose of antigen, the route of exposure, number of exposures, and the genetic background of the host, especially with regard to the major histocompatibility locus-linked H-2 genes called immune response genes (2, 5, 6, 11). We accurately estimated the protein content of each food type and used the same dose. Routes and numbers of exposures were identical as well. It is possible that H-2^b MHC class II gene products of C57Bl/6 may bind and present immunogenic peptides for some foods better than they do for others, and this might account for the difference. However, the effects of immune response genes have been shown to be at their most visible when small peptides are used as immunogens, and we used a food protein extract containing a very large number of complex proteins (10, 11). Furthermore, we have found a similar immunogenicity pattern for these food types in another inbred mouse strain with a different H-2 haplotype (unpublished data). Thus, differences in the immunogenicity levels of these food types appear to be related to intrinsic properties associated with different food types. Since the immune response is likely a result of inherent immunogenicity together with exposure, the results may have differed with other exposure protocols. Furthermore, since we injected groups of mice with proteins from multiple food types, the results may also differ if one food type is used at a time.

In a previous study examining antibody responses to soybeans, it was found that rats must be kept on soy-free diets for several generations before an immune response is raised upon soy challenge (5). The results of the present study involving mice that were on a regular diet, for which soybeans elicited barely detectable antibody responses, is consistent with this previous report.

In the present study, we determined food-specific IgG1 antibody serum levels to measure immunogenicity levels of proteins, which were the focus of this study. Since foods tested here are known to be different with regard to their allergenicity levels in humans and since allergic diseases are immune mediated, we reasoned that commonly allergenic foods are more immunogenic than rarely allergenic or nonallergenic foods.

Our results for the primary response demonstrate that tree nuts are somewhat more immunogenic than other allergenic foods. We used raw nut extracts in this study. Nuts are generally consumed roasted in the West, and roasting has been shown to enhance immunogenicity somewhat (1, 9). Furthermore, IgG1 has been shown to mediate active anaphylaxis in the absence of IgE in IgE gene knockout mice (13). Thus, this study's finding that peanuts and tree nuts elicit dramatically strong IgG1 antibody responses in mice may be relevant to anaphylactic responses that are largely linked to peanuts and tree nuts.

For rarely allergenic and nonallergenic foods, whereas spinach induced a robust primary antibody response, sweet potatoes, cherries, and lettuce induced significant antibody responses. These observations need to be understood in the context of human consumption of these foods. Sweet po-

tatoes are usually consumed boiled, and boiling has been shown to reduce immunogenicity (1, 7). In the present study, we used a raw sweet potato extract (Greer Labs) that is used for allergy skin testing in humans. Therefore, when sweet potatoes are consumed boiled, it is possible that they may not retain much immunogenicity. In contrast, spinach, cherries, and lettuce are often consumed raw. Therefore, the ability of the proteins derived from these foods to resist the acid pH and the digestive enzymes in the gut might ultimately determine whether they would be immunogenic for humans.

We are not aware of any previous reports comparing the relative immunogenicity levels of these foods *in vivo*. There are earlier studies on the development of food allergy models for some food types, and some studies have examined relative immunogenicity levels of food types in BALB/c mice. In these studies, either single food types were used or a set of food types different from the one used in this study was used for comparison (3, 7, 8). Furthermore, there are some animals (such as the brown Norway rat or the C3H mouse) that exhibit IgE antibody responses to certain foods such as eggs, milk, and peanuts (4, 7, 8). It remains to be investigated whether commonly allergenic foods differ from rarely allergenic or nonallergenic foods with regard to the IgE antibody response they elicit in rodent models as they do for humans.

In summary, the results of this study indicate that food types vary widely with regard to their immunogenicity levels and that relative immunogenicity does not distinguish commonly allergenic foods from rarely allergenic or non-allergenic foods.

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