

Genetic susceptibility to food allergy is linked to differential T_H2 - T_H1 responses in C3H/HeJ and BALB/c mice

Vivian Morafo, PhD,* Kamal Srivastava, MPhil,* Chih-Kang Huang, MS,
Gary Kleiner, MD, PhD, Soo-Young Lee, MD, Hugh A. Sampson, MD, and
Xiu-Min Li, MD *New York, NY*

Background: Although food allergy is a serious health problem in westernized countries, factors influencing the development of food allergy are largely unknown. Appropriate murine models of food allergy would be useful in understanding the mechanisms underlying food allergy in human subjects.

Objective: We sought to determine the susceptibility of different strains of mice to food hypersensitivity.

Methods: C3H/HeJ and BALB/c mice were sensitized to cow's milk (CM) or peanut by means of intragastric administration, with cholera toxin as a mucosal adjuvant. Mice were then challenged with CM or peanut. Antigen-specific IgE levels, anaphylactic symptoms, plasma histamine levels, and splenocyte cytokine profiles of these 2 strains were compared.

Results: CM-specific IgE levels were significantly increased only in the C3H/HeJ strain, 87% of which exhibited systemic anaphylactic reactions accompanied by significantly increased plasma histamine levels in response to challenge. BALB/c mice exhibited no significant CM-specific IgE response, increased plasma histamine levels, or anaphylactic symptoms. After peanut challenge, 100% of peanut-sensitized C3H/HeJ mice exhibited high levels of peanut-specific IgE and anaphylactic symptoms. In contrast, no hypersensitivity reactions were detected in BALB/c mice, despite the presence of significant serum peanut-specific IgE levels. Splenocytes from CM- and peanut-sensitized C3H/HeJ mice exhibited significantly increased IL-4 and IL-10 secretion, whereas splenocytes from BALB/c mice exhibited significantly increased IFN- γ secretion. **Conclusion:** Induction of food-induced hypersensitivity reactions in mice is strain dependent, with C3H/HeJ mice being susceptible and BALB/c mice being resistant. This strain-dependent susceptibility to food allergy is associated with differential T_H2 - T_H1 responses after intragastric food allergen sensitization. (*J Allergy Clin Immunol* 2003;111:1122-8.)

Key words: Murine strains, food allergy susceptibility, T_H2 - T_H1 cytokines

Abbreviations used

CM: Cow's milk
CMA: Cow's milk allergy
CT: Cholera toxin
PNA: Peanut allergy
TLR4: Toll-like receptor 4

Food allergy, which affects approximately 6% to 8% of children less than 3 years of age and approximately 2% of the US population,¹ is now the leading cause of anaphylactic reactions treated in hospital emergency departments in westernized countries.² Because of the prevalence of food allergy and the frequency and severe consequences of food-induced anaphylactic reactions, food allergy research is receiving increased attention. However, the factors responsible for the development of food allergy remain unknown.

It is estimated that the human gastrointestinal tract processes up to 2 tons of food during a lifetime,³ and although the majority of persons experience no adverse reactions (oral tolerance), food allergy develops in a small percentage of the population. Although it is thought that genetic susceptibility plays a role in the development of food allergy and Sicherer⁴ found a significantly higher rate of concordance for peanut allergy among monozygotic twins, the genetic factors related to the development of food allergies have not been elucidated. Identification of genetic factors predisposing to allergic diseases is notoriously difficult because of differences in diagnostic criteria used to define the allergic phenotypes, variable onset of disease, causative heterogeneity, heterogeneity of environmental factors, and differences in ethnic background.⁵ In addition, it is virtually impossible to prospectively study the development of food allergy in human subjects.⁶

It has long been recognized that there is great similarity between human and murine immune systems in most of the important mechanisms,⁷ such as T_H1 and T_H2 responses,^{8,9} involved in allergic responses. In mice an allergic phenotype can be created under controlled environmental conditions and within defined genetic backgrounds, and because there is a large variety of homozygous inbred strains, cross-breeding allows segregation analyses of polygenic traits. In addition, there are a large number of immunologic reagents available to study the

From the Department of Pediatrics, Mount Sinai School of Medicine, New York.

*These authors contributed equally to this work.

Supported by National Institutes of Health/National Institute of Allergy and Infectious Disease grant P01-AI44236-01.

Received for publication November 27, 2002; revised February 5, 2003; accepted for publication February 14, 2003.

Reprint requests: Xiu-Min Li, MD, Pediatric Allergy and Immunology, The Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029-6574.

© 2003 Mosby, Inc. All rights reserved.

0091-6749/2003 \$30.00 + 0

doi:10.1067/mai.2003.1463

murine immune response, and increasing numbers of knock-out and transgenic strains provide valuable tools to isolate various immunologic responses. These features make murine models particularly attractive for the study of genetic factors involved in allergic disorders. Although passive cutaneous anaphylactic reactions have been used to study differences between different strains of mice in susceptibility to allergy,⁷ strain differences in susceptibility to food allergy have not been defined. Determining genetic contributions to food allergy susceptibility in different strains of mice by using intragastric sensitization and challenge, which mimics human food allergy, should be extremely useful in facilitating our understanding of food allergy in human subjects.

Food allergy is defined as adverse reactions to food caused by immunologic mechanisms.¹⁰ We previously generated murine models of IgE-mediated cow's milk allergy (CMA) and peanut allergy (PNA) by using C3H/HeJ mouse intragastric sensitization and challenge, an effective mucosal adjuvant, and optimal antigen sensitization and challenge doses.^{11,12} In these models intragastric challenge of sensitized mice provoked anaphylactic symptoms and histamine release. Mice with CMA and PNA also exhibited significantly increased antigen-specific serum IgE levels. These models mimic human CMA and PNA both physiologically and immunologically. In our initial studies for generating a murine model of CMA, we tested several strains of mice, including BALB/c, AKR/J, CBA/J, and C3H/HeJ. Unlike C3H/HeJ mice, we were unable to induce CMA in BALB/c or AKR/J mice and induced only a minimal reaction in CBA/J mice after the same cow's milk (CM) sensitization-challenge regimen used for C3H/HeJ mice (data not shown), showing variable genetic susceptibility to food allergy in mice, as in human subjects. In the present study we focused on C3H/HeJ and BALB/c mice to further characterize the susceptibility to food allergy because previous publications¹¹⁻¹³ and our preliminary study showed that C3H/HeJ mice were sensitive to food allergy, but BALB/c mice, a commonly used strain in models of allergic diseases induced by systemic antigen sensitization, were not.^{14,15} We compared the susceptibility of these 2 strains to induction of CMA, the most common food allergy in human subjects, and PNA, which provokes the most severe reactions in human subjects, and found that C3H/HeJ mice had CMA and PNA reactions, whereas BALB/c mice exhibited no hypersensitivity reactions when subjected to the same CM and peanut sensitization-challenge regimens. To determine the immunologic mechanisms underlying this strain-dependent susceptibility to food allergy, we measured splenocyte cytokine production and found that IL-4 and IL-10, but not IFN- γ production was significantly increased in C3H/HeJ mice, whereas IFN- γ but not IL-4 or IL-10, production was significantly increased in BALB/c mice. These results suggest that genetic variables, which appear to be at least in part caused by differential cytokine responses, influence the development of food allergy.

METHODS

Animals and reagents

Three- to 5-week-old mice purchased from Jackson Laboratories were housed in a pathogen-free environment in the animal facility at Mount Sinai School of Medicine. Guidelines for the care and use of the animals were followed ("Guide for the Care and Use of Laboratory Animals," National Institute of Health publication no. 86-23, as revised).¹⁶

Homogenized CM and ground whole peanut were used for sensitization, as described previously.^{11,12} Purified CM proteins (Ross Laboratories) and crude peanut extract were prepared as described previously¹⁷ and used for ELISA and in vitro cell cultures. Cholera toxin (CT) was purchased from List Biological Laboratories, Inc. dinitrophenyl conjugated with BSA was purchased from Sigma. Polyclonal antibodies (sheep anti-mouse IgE and biotinylated donkey anti-sheep IgG) for IgE measurement by means of ELISA were purchased from the Binding Site, Inc. ELISA kits for cytokine measurements were purchased from Pharmingen. Anti-dinitrophenyl IgE was purchased from Accurate Scientific, Inc.

Antigen sensitization and challenge by means of oral administration

Three-week-old female C3H/HeJ and BALB/c mice ($n = 5-9$ per group) were intragastrically sensitized with homogenized CM (equivalent to 1 mg/g body weight CM proteins)-CT adjuvant (0.3 μ g/g body weight), as described previously,¹¹ and boosted weekly for 5 weeks. Six weeks after the initial sensitization, all mice were challenged intragastrically with homogenized CM equivalent to 33 mg per mouse of CM proteins.

We previously found that peanut is more allergenic than CM, regardless of dose, because sensitization of mice less than 3 weeks old is required to generate CMA, whereas 5-week-old C3H/HeJ mice can be sensitized to peanut and had PNA after a 5-week sensitization-challenge protocol.¹² In a preliminary study this protocol did not induce anaphylactic reactions in BALB/c mice but did induce peanut-specific IgE (unpublished data). In this study we used a more rigorous protocol for peanut sensitization. Briefly, 5-week-old C3H/HeJ and BALB/c mice ($n = 5-9$ per group) were sensitized intragastrically with 10 mg of equivalent peanut protein with CT (20 μ g per mouse) in 0.5 mL of PBS-containing 1.5% NaHCO_3 and boosted 5 times with the sensitization dose at weekly intervals and an additional 2 boostings at 2-week intervals with 50 mg of equivalent protein-CT. Mice were challenged intragastrically with 50 mg of equivalent peanut protein at week 14 after the initial sensitization. Mice were fasted for 2 hours before each oral sensitization and boosting and challenge. Naive mice served as control animals.

Measurement of systemic anaphylactic responses and plasma histamine levels

Systemic anaphylactic reactions were scored from 0 to 5 according to symptoms observed 30 minutes after CM or peanut challenge, as described previously.^{11,12} Blood was collected 30 minutes after the second intragastric challenge. Plasma was prepared as previously described.^{11,18} Plasma histamine levels were measured by using a Histamine Immunoassay kit (Immunotech, Beckman Coulter Company) according to the manufacturer's protocol. All assays were performed in duplicate, and histamine standards were run in duplicate.

Measurement of antigen-specific serum IgE

Blood was drawn at times of sensitization-boosting and 1 day before challenge. Sera were frozen at -80°C until analyzed. CM- and peanut-specific IgE levels were determined by means of ELISA, as described previously.^{11,12}

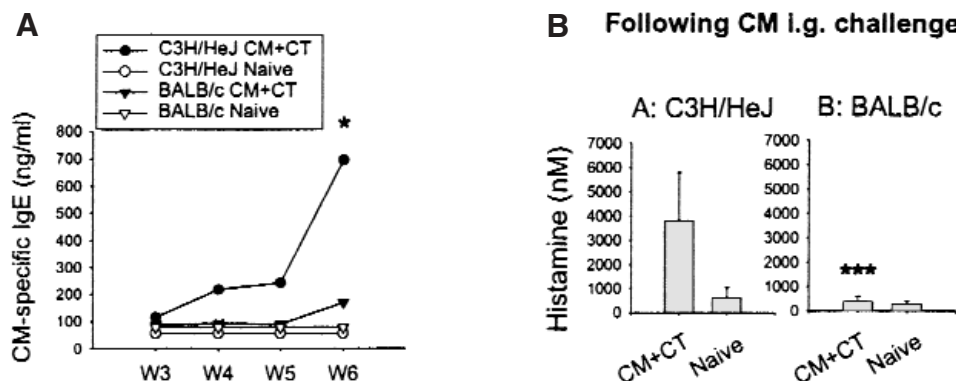


FIG 1. A, Levels of serum CM-specific IgE. Sera from each group were collected at several time points after CM-CT sensitization. CM-specific IgE levels were determined by means of ELISA. Data are presented as means $\pm$ SEM of each group of mice ($n = 5-9$). * $P < .05$ versus BALB/c. **B,** Plasma histamine levels. Plasma was obtained 30 minutes after CM challenge, and plasma histamine levels were determined by using an enzyme immunoassay kit. Data are presented as means $\pm$ SEM. *** $P < .001$ versus BALB/c mice.

TABLE I. Induction of CMA reactions after CM oral challenge

Strain	Sensitization (intragastric)	Challenge (intragastric)	No. of anaphylactic reactions (%)	Score (median)
C3H/HeJ	CM + CT	CM	7/9 (78)	1.8
C3H/HeJ	—	CM	0/5 (0)	—
BALB/c	CM + CT	CM	0/5 (0)	—
BALB/c	—	CM	0/5 (0)	—

Spleen cell cultures and cytokine level measurements

Thirty minutes after challenge and recording of anaphylactic response scores, mice were killed, and spleens from each group were removed and pooled. Single splenocytes were prepared by mincing the spleens with 2 sterile slides (Fisher Scientific), lysing of RBCs with lysing buffer (Sigma Chemical Co), and passing through a cell strainer (Becton Dickinson and Co). After extensive washing, cells were suspended in complete cell culture medium (RPMI 1640 containing 10% FCS and 1% penicillin-streptomycin). Splenocytes (4×10^6 /mL per well) from CM-sensitized mice and naive mice were cultured in 24-well plates in the presence or absence of purified CM proteins (200 μ g/mL), and supernatants were collected 48 hours later. Splenocytes (4×10^6 /mL per well) from peanut-sensitized mice and naive mice were cultured in 24-well plates in the presence or absence of crude peanut extract (200 μ g/mL), and supernatants were collected as described previously.^{11,18,19} Cytokines were measured by using an ELISA kit according to the manufacturers' instructions.

Statistical analysis

The Student *t* test was performed for comparison between 2 groups (C3H/HeJ vs BALB/c mice or sensitized-challenged mice vs naive mice) if the data passed the normality test. The Mann-Whitney rank sum test was used to analyze the difference between 2 groups (C3H/HeJ vs BALB/c mice or sensitized-challenged mice vs naive mice) if the data failed the normality test. We computed the required sample per group; for 80% power, by using a 2-tailed test at the 0.05 level on the basis of our preliminary study, 4 to 5 mice per group were required. Data are presented as means and SEM. *P* values of less than .05 were considered statistically significant.

RESULTS

Induction of CM-specific IgE synthesis and CMA reactions by means of oral sensitization and challenge

After oral CM-CT sensitization, CM-specific IgE levels were monitored kinetically. CM-specific IgE levels were markedly increased in C3H/HeJ mice compared with in naive mice at the time of challenge at week 6. CM-specific IgE levels in BALB/c mice, however, were only slightly greater than those of naive mice (Fig 1, A).

Because increased plasma histamine levels reflect mast cell degranulation and are one of the major mediators of anaphylactic reactions, we measured plasma histamine levels after oral CM challenge. Plasma histamine levels were increased in sensitized C3H/HeJ mice (Fig 1, B) but not in BALB/c mice, in which plasma histamine remained at baseline levels (Fig 1, B).

Anaphylactic reactions after ingestion of allergenic foods are the hallmarks of immediate food hypersensitivity. CM-sensitized C3H/HeJ and BALB/c mice and naive mice were challenged with CM to evaluate the influence of genetic background on hypersensitivity reactions. Anaphylactic reactions were observed in 87% of C3H/HeJ mice (median score, 1.8; Table I). In contrast, no reactions were observed in CM-sensitized BALB/c mice or naive mice of either strain. The anaphylactic reactions in C3H/HeJ mice were associated with increased plasma histamine levels. These results demon-

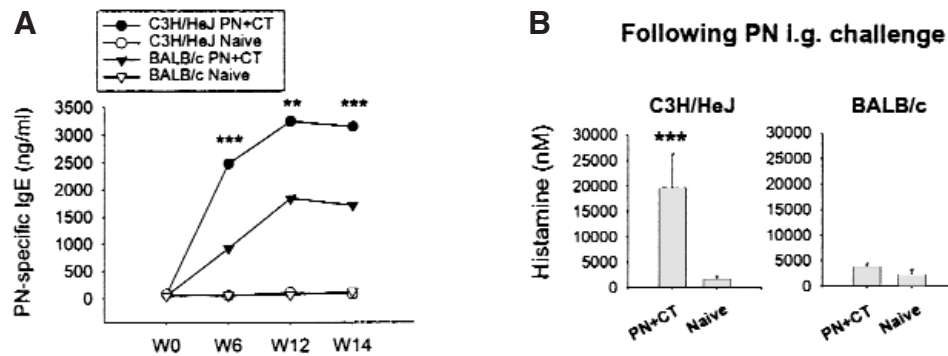


FIG 2. A, Levels of serum peanut-specific IgE. Sera from each group were collected at several time points after peanut-CT sensitization. Peanut-specific IgE levels were determined by means of ELISA. Data are presented as means $\pm$ SEM of each group of mice ($n = 5-9$). $**P < .01$ and $***P < .001$ versus BALB/c mice. **B**, Plasma histamine levels. Plasma was obtained 30 minutes after peanut challenge, and plasma histamine levels were determined by using an enzyme immunoassay kit. Data are presented as means $\pm$ SEM. $***P < .001$ versus BALB/c mice.

TABLE II. Induction of PNA reactions after peanut oral challenge

Strain	Sensitization (intragastric)	Challenge (intragastric)	No. of anaphylactic reactions (%)	Score (median)
C3H/HeJ	Peanut + CT	Peanut	9/9 (100)	2.7
C3H/HeJ	—	Peanut	0/9 (0%)	—
BALB/c	Peanut + CT	Peanut	0/5 (0%)	—
BALB/c	—	Peanut	0/5 (0%)	—

strate that C3H/HeJ mice, but not BALB/c mice, had CMA in response to CM sensitization-challenge.

Induction of peanut-specific IgE synthesis and PNA reactions by means of oral sensitization and challenge

Because peanut is more allergenic than CM in murine models of food-induced hypersensitivity,²⁰ we next determined whether BALB/c mice could be sensitized with the more allergenic food protein peanut by use of a more rigorous sensitization-boosting protocol. We found that peanut-specific IgE levels were markedly increased at week 6 after peanut sensitization-boosting in C3H/HeJ mice (Fig 2, A). Additional boosting further increased peanut-specific IgE in C3H/HeJ mice, which peaked at week 12. IgE levels were also increased at week 6 in BALB/c mice and further increased after this more rigorous sensitization-boosting protocol, but they were significantly lower than in C3H/HeJ mice at each time point ($P < .001$ at weeks 6 and 14 and $P < .01$ at week 12 vs levels in BALB/c mice).

Histamine levels were markedly increased after peanut challenge of peanut-sensitized C3H/HeJ mice ($P < .001$; Fig 2, B). As expected, 100% of C3H/HeJ mice had anaphylactic symptoms (median score, 2.8) after intragastric peanut challenge (Table II). In contrast, despite significant levels of peanut-specific IgE at the time of challenge at week 14, plasma histamine levels in BALB/c mice remained at baseline levels (Fig 2, B), and no symptoms were observed in BALB/c mice (Table II). These results

demonstrated that BALB/c mice did not have PNA reactions, even when a strongly allergenic protein was administered and a more rigorous sensitization protocol was used.

Cytokine responses to CMA and PNA sensitization protocols

IL-4 levels were significantly increased in CM-sensitized C3H/HeJ splenocyte culture supernatants but only negligibly increased in supernatants of splenocyte cultures from BALB/c mice ($P < .001$; Fig 3, A). In contrast, IFN- γ levels were negligible in C3H/HeJ splenocyte cultures but markedly increased in BALB/c cultures ($P < .001$; Fig 3, B). IL-4 and IFN- γ production by peanut-sensitized C3H/HeJ and BALB/c splenocytes followed the same pattern as CM-sensitized C3H/HeJ and BALB/c splenocytes (Fig 3, D and E); that is, IL-4 production by C3H/HeJ splenocyte cultures was significantly higher and IFN- γ production was significantly lower than in splenocytes from BALB/c mice. We also measured IL-10, a classical T_H2 cytokine, which is also believed to be involved in induction of oral tolerance²¹ and downregulation of the allergic response.²² We found that splenocytes from CM- and peanut-sensitized C3H/HeJ mice released significantly more IL-10 than splenocytes from BALB/c mice ($P < .01$; Fig 3, C and F). Therefore it appeared that increased IL-10 was associated with induction of CMA and PNA, increased IL-4 production, and decreased IFN- γ production in this model. These findings are in accord with those of a recent study, which also found increased IL-10 levels in C3H/HeJ mice after intragastric peanut sensitization.¹³

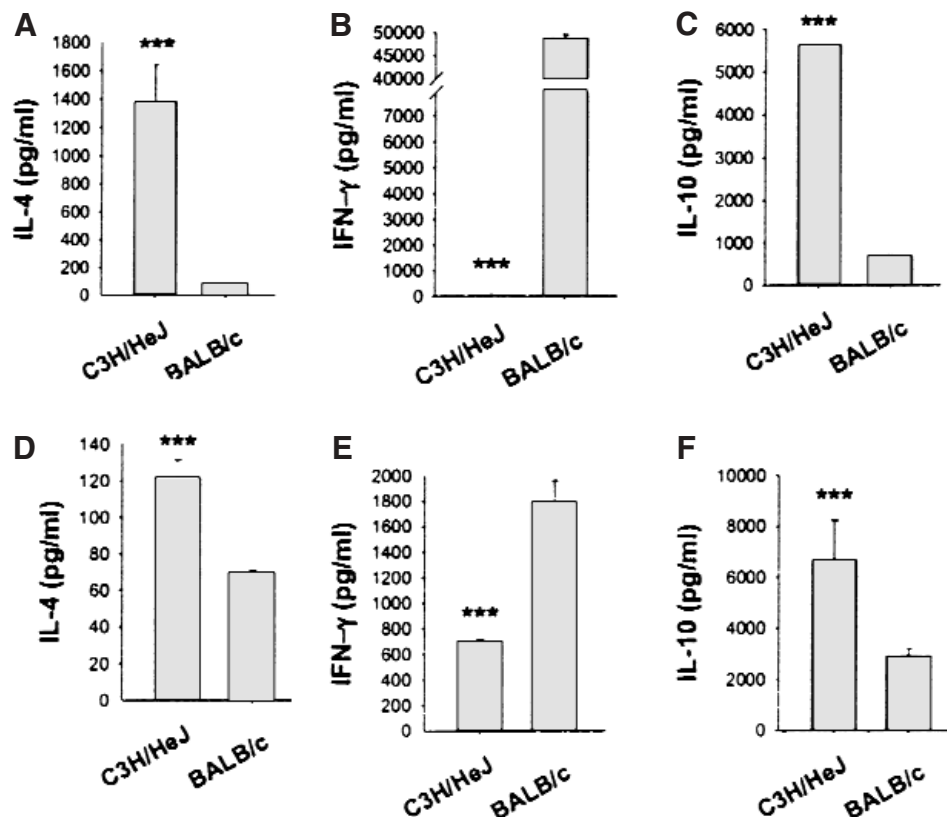


FIG 3. Cytokine levels in splenocyte culture supernatants. Immediately after evaluation of anaphylactic reactions, splenocytes were isolated from each group of mice ($n = 5$) and cultured in the presence of specific antigen. Cells cultured in medium alone or concanavalin A served as negative and positive controls (data not shown). CM-induced IL-4, IFN- γ and IL-10 levels (A-C) and peanut-induced IL-4, IFN- γ and IL-10 levels (D-F) were measured by means of ELISA. Data are presented as means $\pm$ SEM. *** $P < .001$ versus BALB/c mice.

DISCUSSION

In this study we determined susceptibility of C3H/HeJ and BALB/c mice to induction of CMA and PNA. C3H/HeJ mice had significant levels of antigen-specific IgE after sensitization and high levels of plasma histamine accompanied by anaphylactic reactions in response to challenge with CM. In contrast, BALB/c mice showed only a slight CM-specific IgE response to CM sensitization, and neither increased plasma histamine levels nor anaphylactic symptoms were induced by CM challenge of this strain. These findings demonstrate that induction of CMA in mice is strain dependent, with C3H/HeJ being a high-responder strain and BALB/c being a nonresponder strain. We also found that BALB/c mice sensitized with peanut, a more allergenic food allergen, by using a more rigorous sensitization protocol, did have a significant peanut-specific serum IgE response but showed no allergic symptoms or increase in plasma histamine levels after oral peanut challenge. Taken together, these results suggest that under the same experimental conditions, genetic background plays a critical role in the development of food hypersensitivity reactions.

T_H2 cytokines are central to the pathogenesis of allergic disorders.²³ A T_H2 -skewed response has been observed in food allergy. Schade et al,²⁴ for example, recently reported that T-cell clones generated from infants with CMA produced high levels of IL-4, IL-5, and IL-13 and low levels of IFN- γ whereas infants without CMA produced high levels of IFN- γ and low levels of IL-4, IL-5, and IL-13. Peanut-specific T_H2 clones have also been generated from patients with PNA.^{25,26} The differences in susceptibility to food hypersensitivity found in this study were accompanied by differences in splenocyte cytokine secretion. Splenocytes from C3H/HeJ mice, which had CMA and PNA responses, produced more IL-4 and IL-10 and less IFN- γ than BALB/c mice, which did not have CMA or PNA. These differential T_H2 - T_H1 responses to ingested food proteins might be responsible for the differential susceptibility to food allergy, with a T_H2 -dominant response linked to food allergy in the susceptible strain and a T_H1 response linked to tolerance in the resistant strain.

It has been suggested that the normal human intestinal immune system preferentially responds to various food antigens with a dominant T_H1 profile.^{27,28} BALB/c mice

exhibited a predominant T_H1 cytokine (IFN- γ) response that could have inhibited T_H2 -mediated food hypersensitivity reactions. A previous study found that feeding BALB/c mice with OVA induced IFN- γ and oral tolerance and that depletion of IFN- γ impaired the induction of oral tolerance.¹⁵ On the other hand, less IFN- γ induction by C3H/HeJ mice might have resulted in failure to control T_H2 cell responses to milk or peanut allergens, thereby resulting in the development of food allergy.

IL-10, initially characterized as a T_H2 cytokine²⁹ that suppressed IFN- γ and IL-12 secretion³⁰ and inflammatory responses in autoimmune diseases,²¹ has also been found to be associated with induction of antigen-induced pulmonary inflammation in animal models of allergic asthma³¹ and in asthmatic patients.³² In contrast, IL-10 was recently reported to suppress T_H2 -induced allergic inflammatory processes.^{22,33} We therefore hypothesized that IL-10 suppression of T_H2 cytokines might also be involved in suppressing the induction of food allergy in BALB/c mice. However, we found that C3H/HeJ splenocytes secreted significantly more IL-10 than BALB/c splenocytes, suggesting that IL-10 is associated with induction of T_H2 -mediated food allergy rather than suppression of food allergy, perhaps by means of its known suppression of IFN- γ production. A recent study showed that production of IL-10 and other T_H2 cytokines (IL-4, IL-5, and IL-13) was increased by splenocytes from IFN- $\gamma^{-/-}$ mice.³⁴ These results suggest that susceptibility to food allergy, like other allergies, is due to an imbalance of the T_H1 - T_H2 immunoregulatory mechanisms. Further research is necessary to confirm a critical role for IL-4, IL-10, or IFN- γ and other cytokines in induction or suppression of food allergy in these strains.

The mechanisms underlying genetic predisposition toward T_H2 or T_H1 responses linked to food allergy in the susceptible strain or food tolerance in the resistant strain have not been elucidated. C3H/HeJ mice lack functional toll-like receptor 4 (TLR4) and do not respond to LPS.³⁵ Although LPS induces IL-12 and IFN- γ production by dendritic cells,³⁶ the role of LPS in allergy is controversial. It was reported that LPS administration to rodents prevented development of T_H2 responses and pulmonary inflammation, as well as airway hyperresponsiveness.³⁷ LPS exposure has also been associated with a decreased risk of aeroallergen sensitization in human subjects.³⁸ However, LPS exposure has recently been associated with an increased risk of asthma-like respiratory symptoms in human subjects.³⁹ Furthermore, TLR4 has been found to be necessary for the optimal development of T_H2 responses rather than T_H1 responses.⁴⁰ In addition, there is no correlation between the absence of LPS responsiveness and the development of oral tolerance. Mowat et al⁴¹ demonstrated induction of oral tolerance to OVA in C3H/HeJ mice in an early study. We also found that C3H/HeJ mice have oral tolerance to CM when fed high doses or sensitized after 8 weeks of age.⁴² Furthermore, we have investigated the possible relationship between TLR4 and sensitivity to food allergy by using congenic BALB/c mice (C. C3H Tlr^{ps-d}), which express

the C3H/HeJ gene sequence responsible for defective function of TLR4 in C3H/HeJ mice,⁴³ and found that C. C3H Tlr^{ps-d} mice are also not susceptible to symptomatic peanut allergy (Berin et al, unpublished data). Therefore we conclude that TLR4 is unlikely to play a significant role in susceptibility or tolerance to CMA or PNA in C3H/HeJ and BALB/c mice. Thus other mechanisms, such as differing properties of intestinal antigen-presenting cells in the processing food allergens, might determine predisposition to T_H2 responses and food allergy of C3H/HeJ mice.

A particularly interesting finding in this study was the absence of hypersensitivity symptoms in BALB/c mice after oral peanut challenge, despite the presence of increased serum peanut-specific IgE levels. This finding that increased allergen-specific serum IgE levels was not predictive of hypersensitivity reactions mirrors clinical findings in human subjects, in whom there is no clear correlation between IgE levels and the severity of symptoms in patients with peanut allergy.^{44,45} The reasons for this are unknown but might be due to undefined factors, such as IFN- γ inhibition of mast cell–basophil mediator release on re-exposure to antigen, as shown in vitro.^{46,47} The findings of strain differences in both susceptibility to oral sensitization and hypersensitivity reactions after challenge of sensitized mice make these murine model systems valuable tools to facilitate studies aimed at understanding the genetic influences of food allergy in human subjects. Although much work is needed to elucidate the mechanisms underlying food allergy, the findings of this study raise questions regarding the use of systemic sensitization and systemic challenge methods to investigate food allergy in animal models. Development of food protein–specific IgE is not in itself food allergy, and although the inherent immunologic and toxicologic properties of food proteins can be addressed by systemic administration, food allergy investigations should use both gastrointestinal sensitization and challenge of appropriate animal strains.

In conclusion, development of food allergy in mice is strain dependent. Under the conditions used in this study, C3H/HeJ mice were susceptible to CMA and PNA, whereas BALB/c mice were not. Although the mechanisms underlying genetic predisposition to food allergy are unknown, our results suggest that genetic predispositions toward a dominant T_H2 response or dominant T_H1 response after antigen ingestion might be linked to the susceptibility and resistance to food allergy.

REFERENCES

1. Sampson HA. Food allergy. Part 1: immunopathogenesis and clinical disorders. *J Allergy Clin Immunol* 1999;103:717-28.
2. Sampson HA. Clinical practice. Peanut allergy. *N Engl J Med* 2002;346:1294-9.
3. Johansson SG, Danneus A, Lilja G. The relevance of anti-food antibodies for the diagnosis of food allergy. *Ann Allergy* 1984;53:665-72.
4. Sicherer SH. Clinical update on peanut allergy. *Ann Allergy Asthma Immunol* 2002;88:350-61.
5. Ruffilli A, Bonini S. Susceptibility genes for allergy and asthma. *Allergy* 1997;52:256-73.

6. Helm RM. Food allergy animal models: an overview. *Ann N Y Acad Sci* 2002;964:139-50.
7. Daser A, Batjer N, Kolsch U, Kotz K, Schmeling H, Schou C, Renz H. Quantitative assessment of immediate cutaneous hypersensitivity in a model of genetic predisposition to atopy. *Int Arch Allergy Immunol* 1998;117:239-43.
8. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986;136:2348-57.
9. Romagnani S. Human TH1 and TH2 subsets: doubt no more. *Immunol Today* 1991;12:256-7.
10. Bjorksten B. The epidemiology of food allergy. *Curr Opin Allergy Clin Immunol* 2001;1:225-7.
11. Li XM, Schofield BH, Huang CK, Kleiner GA, Sampson HA. A murine model of IgE mediated cow milk hypersensitivity. *J Allergy Clin Immunol* 1999;103:206-14.
12. Li XM, Serebrisky D, Lee SY, Huang CK, Bardina L, Schofield BH, Stanley JS, et al. A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses. *J Allergy Clin Immunol* 2000;106:150-8.
13. Bashir ME, Andersen P, Fuss IJ, Shi HN, Nagler-Anderson C. An enteric helminth infection protects against an allergic response to dietary antigen. *J Immunol* 2002;169:3284-92.
14. Atherton KT, Dearman RJ, Kimber I. Protein allergenicity in mice: a potential approach for hazard identification. *Ann N Y Acad Sci* 2002;964:163-71.
15. Lee HO, Miller SD, Hurst SD, Tan LJ, Cooper CJ, Barrett TA. Interferon gamma induction during oral tolerance reduces T-cell migration to sites of inflammation. *Gastroenterology* 2000;119:129-38.
16. Institute of Laboratory Animal Resources Commission of Life Sciences NRC. Guide for the care and use of laboratory animals. Washington, DC: National Academy Press; 1996.
17. Burks AW, Williams LW, Connaughton C, Cockrell G, O'Brien TJ, Helm RM. Identification and characterization of a second major peanut allergen, Ara h II, with use of the sera of patients with atopic dermatitis and positive peanut challenge. *J Allergy Clin Immunol* 1992;90:962-9.
18. Li X, Huang CK, Schofield BH, Burks AW, Bannon GA, Kim KH, et al. Strain-dependent induction of allergic sensitization caused by peanut allergen DNA immunization in mice. *J Immunol* 1999;162:3045-52.
19. Lee SY, Huang CK, Zhang TF, Schofield BH, Burks AW, Bannon GA, et al. Oral administration of IL-12 suppresses anaphylactic reactions in a murine model of peanut hypersensitivity. *Clin Immunol* 2001;101:220-8.
20. Maitland AL, Lee J, Srivastava K, Burks AW, Bannon GA, Sampson HA, et al. Peanut is more allergenic than cow's milk in murine models of food-induced hypersensitivity [abstract]. *J Allergy Clin Immunol* 2001;107(suppl):S140.
21. Strobel S. Oral tolerance, systemic immunoregulation, and autoimmunity. *Ann N Y Acad Sci* 2002;958:47-58.
22. Barnes PJ. IL-10: a key regulator of allergic disease. *Clin Exp Allergy* 2001;31:667-9.
23. Romagnani S. The role of lymphocytes in allergic disease. *J Allergy Clin Immunol* 2000;105:399-408.
24. Schade RP, Ieperen-Van Dijk AG, Van Reijssen FC, Versluis C, Kimpen JL, Knol EF, et al. Differences in antigen-specific T-cell responses between infants with atopic dermatitis with and without cow's milk allergy: relevance of TH2 cytokines. *J Allergy Clin Immunol* 2000;106:1155-62.
25. de Jong EC, Van Zijverden M, Spanhaak S, Koppelman SJ, Pellegrom H, Penninks AH. Identification and partial characterization of multiple major allergens in peanut proteins. *Clin Exp Allergy* 1998;28:743-51.
26. Dorion BJ, Burks AW, Harbeck R, Williams LW, Trumble A, Helm RM, et al. The production of interferon-gamma in response to a major peanut allergy, Ara h II correlates with serum levels of IgE anti-Ara h II. *J Allergy Clin Immunol* 1994;93:93-9.
27. Nilsen EM, Jahnsen FL, Lundin KE, Johansen FE, Fausa O, Sollid LM, et al. Gluten induces an intestinal cytokine response strongly dominated by interferon gamma in patients with celiac disease. *Gastroenterology* 1998;115:551-63.
28. MacDonald TT, Pettersson S. Bacterial regulation of intestinal immune responses. *Inflamm Bowel Dis* 2000;6:116-22.
29. Fiorentino DF, Bond MW, Mosmann TR. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J Exp Med* 1989;170:2081-95.
30. Hsu DH, Moore KW, Spits H. Differential effects of IL-4 and IL-10 on IL-2-induced IFN-gamma synthesis and lymphokine-activated killer activity. *Int Immunol* 1992;4:563-9.
31. Lin CC, Lin CY, Ma HY. Pulmonary function changes and increased Th-2 cytokine expression and nuclear factor κ B activation in the lung after sensitization and allergen challenge in brown Norway rats. *Immunol Lett* 2000;73:57-64.
32. Wong CK, Ho CY, Ko FW, Chan CH, Ho AS, Hui DS, et al. Proinflammatory cytokines (IL-17, IL-6, IL-18 and IL-12) and Th cytokines (IFN-gamma, IL-4, IL-10 and IL-13) in patients with allergic asthma. *Clin Exp Immunol* 2001;125:177-83.
33. Oh JW, Seroogy CM, Meyer EH, Akbari O, Berry G, Fathman CG, et al. CD4 T-helper cells engineered to produce IL-10 prevent allergen-induced airway hyperreactivity and inflammation. *J Allergy Clin Immunol* 2002;110:460-8.
34. Lewkowich IP, Hayglass KT. Endogenous IFN-gamma and IL-18 production directly limit induction of type 2 immunity in vivo. *Eur J Immunol* 2002;32:3536-45.
35. Poltorak A, He X, Smirnova I, Liu MY, Huffer CV, Du X, et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 1998;282:2085-8.
36. Heinzel FP, Rerko RM, Ling P, Hakimi J, Schoenhaut DS. Interleukin 12 is produced in vivo during endotoxemia and stimulates synthesis of gamma interferon. *Infect Immun* 1994;62:4244-9.
37. Vannier E, Lefort J, Lellouch-Tubiana A, Terlain B, Vargaftig BB. Lipopolysaccharide from *Escherichia coli* reduces antigen-induced bronchoconstriction in actively sensitized guinea pigs. *J Clin Invest* 1991;87:1936-44.
38. Gereda JE, Leung DY, Thatayatikom A, Streib JE, Price MR, Klinnert MD, et al. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet* 2000;355:1680-3.
39. Park JH, Gold DR, Spiegelman DL, Burge HA, Milton DK. House dust endotoxin and wheeze in the first year of life. *Am J Respir Crit Care Med* 2001;163:322-8.
40. Dabbagh K, Dahl ME, Stepick-Biek P, Lewis DB. Toll-like receptor 4 is required for optimal development of Th2 immune responses: role of dendritic cells. *J Immunol* 2002;168:4524-30.
41. Mowat AM, Thomas MJ, MacKenzie S, Parrott DM. Divergent effects of bacterial lipopolysaccharide on immunity to orally administered protein and particulate antigens in mice. *Immunology* 1986;58:677-83.
42. Morafo V, Huang CK, Lee SY, Kleiner GA, Sampson HA, Li XM. Genetic susceptibility and other factors influencing the induction of cow's milk hypersensitivity [abstract]. *J Allergy Clin Immunol* 2002;109(suppl):S287.
43. Vogel SN, Wax JS, Perera PY, Padlan C, Potter M, Mock BA. Construction of a BALB/c congenic mouse, C3H-Lpsd, that expresses the Lpsd allele: analysis of chromosome 4 markers surrounding the Lps gene. *Infect Immun* 1994;62:4454-9.
44. Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. *J Allergy Clin Immunol* 1997;100:444-51.
45. Hourihane JO, Kilburn SA, Dean P, Warner JO. Clinical characteristics of peanut allergy. *Clin Exp Allergy* 1997;27:634-9.
46. Bissonnette EY, Befus AD. Inhibition of mast cell-mediated cytotoxicity by IFN-alpha/beta and -gamma. *J Immunol* 1990;145:3385-90.
47. Pierkes M, Bellinghausen I, Hultsch T, Metz G, Knop J, Saloga J. Decreased release of histamine and sulfidoleukotrienes by human peripheral blood leukocytes after wasp venom immunotherapy is partially due to induction of IL-10 and IFN-gamma production of T cells. *J Allergy Clin Immunol* 1999;103:326-32.