

Peanut-lupine antibody cross-reactivity is not associated to cross-allergenicity in peanut-sensitized mouse strains

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Abstract

Background: Peanut hypersensitivity is one of the most common food allergies and one of the most common causes of death by food anaphylaxis in children and adults. Cross-reactivity of peanut-specific antibody (Ab) with other legumes is frequently demonstrated but it still remains to be demonstrated whether these responses could lead to clinical signs of cross-allergenicity.

Objective: We sought to evaluate peanut-specific serum IgE and IgG1 antibody (Ab) responses and anaphylactic reaction in mice strains and to assess both cross-reactivity and cross-allergenicity of peanut and lupine.

Methods: Four mice strains (i.e., C3H, BALB/c, CBA and SJL) were sensitized to peanut by intraperitoneal (ip) injection of crude peanut protein extract with alum. Other groups were given oral peanut extract without adjuvant. Peanut-specific antibodies (Abs) and anaphylactic responses to peanut challenge were examined.

Results: The C3H, CBA (H-2^k) and BALB/c (H2-^d) mice exhibited high levels of peanut-specific serum IgE, IgG1 Ab responses after the intra-peritoneal sensitization. Only the two strains of mice in the H-2^k background developed anaphylactic symptoms upon intra-peritoneal challenge with crude peanut protein extract. While cross-reactivity of peanut and lupine was confirmed by ELISA, no clinical symptom of cross-allergenicity was seen after challenge with lupine. Mice that were given oral peanut showed only increase in peanut-specific IgG2a, but no IgE or IgG1 Abs and failed to develop anaphylactic reactions following injection of either peanut or lupine protein.

Conclusion: These results show that mice of different genetic backgrounds can be sensitized to peanut by ip injection to develop anti-peanut Abs that cross react with lupine. In addition, cross-allergenicity may not directly correlate with the presence of cross-reactive Abs since no clinical symptoms of cross-allergenicity was seen after ip challenge with lupine.

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1. Introduction

Peanut is a major food allergen and the most common cause of fatal and near-fatal food-related anaphylaxis [1–2]. The allergy to peanut is characterized by more severe symptoms than other food allergies and by a high rate of symptoms on minimal contact [3,4]. The incidence of peanut, and food allergies in general, has increased in the recent decades. In contrast, little progress was made for prevention and treatment of peanut allergy. Thus, avoidance, the only effective preventive treatment, is extremely difficult because of the widespread and often disguised use of peanuts in the food industry and others [5]. Only a small number of peanut allergic patients carries pharmacologic agent that interfere with the consequences of mediator release (i.e., epinephrine) and even timely injection of this may not prevent death [6,7]. Desensitization by injection of aqueous peanut extracts showed an unfavourable risk–benefit ratio [8]. An anti-IgE antibody therapy was shown to increase the threshold of sensitivity to peanut challenge that could be protective against most accidental ingestion of peanuts [9], although its efficacy against skin and other potential routes of peanut exposure remain to be demonstrated. The genetic and environment factors that lead to abnormal production of IgE to peanut remains poorly understood. In addition, an increasing number of reports suggests that other parameters, including IgG [10] and Th1 cytokines play a role in allergic reactions [11].

The problem of peanut allergy is further enhanced by the possibility of cross-reactive reactions between peanut and the other members of the legume family. In vitro studies have shown that more than one out of three peanut allergic patients possess IgE that react with other legumes [12] and extensive immunologic cross reactivities among legumes have been demonstrated [13]. Several clinical reports suggested that peanut allergic subjects developed allergic reaction upon contact with other legumes including soybean, lens, or lupine. However, only rare clinical cross-reaction were demonstrated by double-blind, placebo-controlled food challenges (DBPCFC) studies [14,15]. In contrast with these studies, others have suggested that the risk of clinical manifestations of allergy following exposure of peanut allergic patients to lupine is higher than with other legumes [16,17].

However, the mechanisms underlying these reactions remain poorly understood.

Mouse models have been extensively used to study human diseases. More specifically, the human and murine immune systems share mechanisms involved in allergic responses. The genetic background plays an important role in human atopy. Murine studies have shown that environment allergic sensitization to peanut allergen is strain-dependent [4]. For example, C3H (H-2^k) mice were shown to be sensitive to food allergy [4,18,19] but not BALB/c (H-2^d) mice [19]. Few mouse studies examined allergic reactions due to cross-reactivity with new food allergens such as lupine. In this regard, a large portion of the European population is now being exposed to lupine since the generalization of its use as an additive to wheat flour. The aim of the present study was to delineate potential lupine-peanut cross-reactivity and cross-allergenicity on peanut-sensitized murine models. For that purpose four strains (C3H, BALB/c, CBA and SJL) of inbred mice with different MHC were sensitized to peanut to address the potential role of other MHC haplotypes as well as other genetic factors on allergic reaction to new food allergens. Serum levels of Abs reacting with peanut and lupine were characterized in vitro as well as in vivo anaphylactic reaction after ip challenge with peanut or lupine.

2. Materials and methods

2.1. Peanut and lupine protein extracts

Sweet lupine proteins extract was prepared by incubating lupine flour (TERRENA, Ancenis, France) in sodium bicarbonate (pH 9.4) under stirring. The protein extract was then dialyzed against PBS, concentrated by filtration and aliquot stored at –70 °C. The peanut protein extract was obtained by using a published method [20] with modifications. Briefly, the kernels (100 g) were ground in a blender and the ground peanuts mixed with 250 ml of acetone (diethyl ether) and stirred for 1 h at 4 °C. The suspension was allowed to settle and the acetone decanted and discarded. The pellet was resuspended in 250 ml of diethyl ether and allowed to settle. This step was repeated five times and the defatted peanut extract was separated from the diethyl ether by aspiration and

air-dried overnight (24 h). An aqueous extract of the defatted peanuts was prepared by adding 300 ml of 0.1 M ammonium bicarbonate adjusted the pH to 8. The mixture was stirred for 20 h at 25 °C. The extract was clarified by centrifugation for 40 min at 48,000 $\times g$ at 4 °C, freeze-dried and stored at -4 °C. Gradients 4–15% sodium dodecyl sulphate–polyacrylamide mini gels (Bio-Rad Laboratories, Hercules, CA) were used to compare protein composition in lupine and peanut extracts. For this purpose, lupine and peanut samples containing 30 μg of proteins were mixed with loading buffer (Tris HCl 1M, glycerol, SDS and 2-mercapto-ethanol) and heated 5 min at 90 °C. Samples and a molecular weight marker were then loaded into the gel and the electrophoresis was performed for 1 h at 100 mV. The protein bands were visualized with 0.1% Coomassie brilliant blue and the images analyzed with the Kodak 1D 3.0 software (Eastman Kodak, Rochester, NY).

2.2. Mice, peanut sensitization and sample collection

The animal protocol complied with NIH guidelines. Mice 6 weeks old from C3H/HeSn (H-2^k), CBA/j (H-2^k), BALB/c (H-2^d), and SJL/J (H-2^s) strains were purchased from Harlan Nerediand France Laboratory. The mice were fed ad libitum a standard laboratory food (cereal, 839 g/kg; vitamins and minerals, 41 g/kg; fish proteins, 40 g/kg; and vegetable proteins, 80 g/kg; Extralabo; APAE-INRA, Jouy en Josas, France). For oral sensitization, mice ($n=10$ per group) received weekly (for 24 h) during three months 3 g of their habitual diet containing 2.5 mg of peanut extract and blood samples were collected every fifteen days. For intra-peritoneal sensitization, mice ($n=10$ per group) received 150 μl of 7 $\mu g/ml$ peanut protein in PBS with Al(OH)₃ as adjuvant. Mice received four injections at 15 days intervals. Blood samples were collected from each group of mice before and after immunization by retro-orbital puncture all along experimentation. After centrifugation, individual sera were collected and stored at -20 °C.

2.3. In vitro antibody reactivity to peanut and lupine

The level of peanut and lupine IgE, IgG, IgG1 and IgG2a response was determined by ELISA.

Microtiter plates (Nunc-maxisorp, France) were coated with peanut or lupine (10 $\mu g/ml$ in PBS pH 7.4). After an overnight at 4 °C, plates were washed and blocked with 3% BSA PBS for 1 h at 37 °C. Fifty (50) μl of serum samples (Diluted in 1% BSA PBS) was added to the plates and incubated 2 h at 37 °C. Plates were then washed, and incubated for 1 h at 37 °C with 50 μl of 2 $\mu g/ml$ biotinylated polyclonal specific antibody for IgG (Sigma B9904, France), or biotinylated monoclonal specific antibodies for IgE, IgG1 and IgG2a (Pharmingen, France; 02232D, 02122D and 02012D, respectively). Fifty (50) μl of streptavidin–peroxydase (1:5000 dilution, Sigma E-2886, France) was then added to the plates and incubated for 30 min at 37 °C. After addition of 50 μl of H₂O₂ (30%, 0.25 ml/l, Sigma, France) associated with OPD (0.5 mg/ml, Sigma, France) in a sodium citrate buffer 0.05 M pH 5.1 used as substrate, the reaction was stopped by adding H₂SO₄ (2N). Between each incubation, the plates were washed with PBS containing 0.01% of tween 20. The absorbance was measured at 490 nm using a microplate reader (Bio-Tek Instruments).

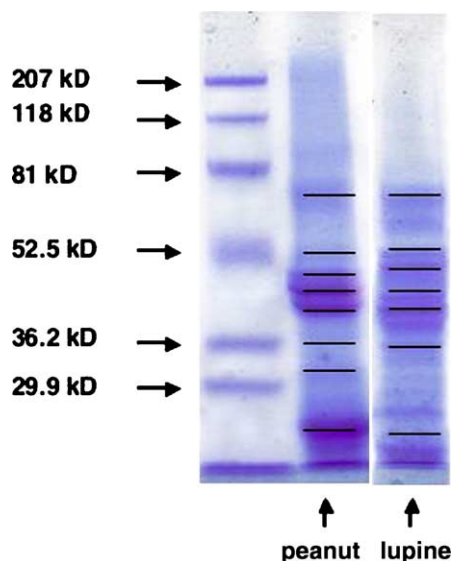


Fig. 1. SDS-PAGE analysis of peanut and lupine protein extracts. The protein bands visualized by Coomassie brilliant blue were analyzed in comparison to a molecular weight marker with a Kodak 1D 3.0 software.

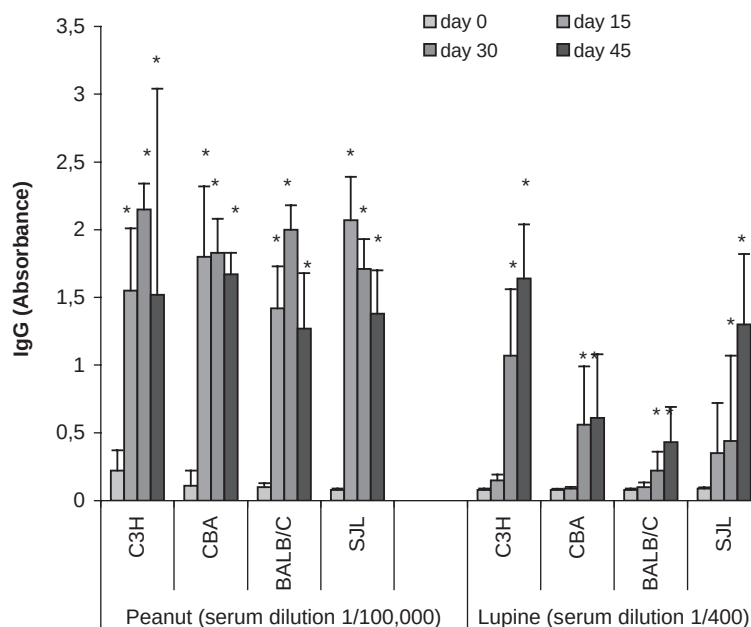


Fig. 2. In vitro reactivity to peanut and lupine antigen of ip peanut-sensitized mice IgG serum antibodies. * Significantly different from day 0 ($P < 0.05$).

2.4. In vivo hypersensitivity response to peanut and lupine

Anaphylactic symptoms of mice were evaluated 40 min after ip challenge with 1 mg of peanut or lupine as

previously reported [4]. The symptoms were quantified by using the following previously reported scoring system [4]: 0, no symptoms; 1, scratching and rubbing around the nose and head; 2, puffiness around the eyes; pillar erect, reduced activity, and/or decreased activity

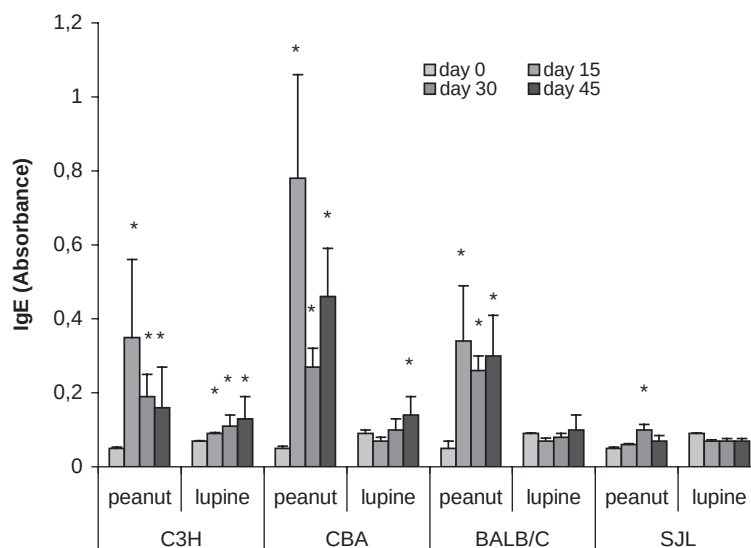


Fig. 3. In vitro reactivity to peanut and lupine of ip peanut-sensitized mice IgE serum antibodies. Serum dilution 1/10. * Significantly different from day 0 ($P < 0.05$).

with decreased activity with increased respiratory rate; 3, wheezing, labored respiration, cyanosis around the mouth and the tail; 4, no activity after prodding, or tremor and convulsion; 5, death.

2.5. Statistical analysis

The statistical significance of the data was determined by *t* test; a value of $p < 0.05$ was considered as significant.

3. Results

A major prerequisite for the development of cross-reactive allergic reaction is the presence of common or cross-reactive antigens (allergens). Thus, we first analyzed the proteins in peanut and lupine extracts to determine to what extent these two members of the botanical family of legumes share common proteins.

As reported in Fig. 1, protein bands with the same molecular weight were detected in peanut and lupine extracts at 19, 22, 46, 50 and 76 kDa. The studies described below determined the potential immunological consequences of the shared proteins in mouse responses to lupine after sensitization with peanut proteins.

3.1. Peanut promotes distinct patterns of peanut-specific antibody responses in mouse strains

Most mouse model of peanut allergy involve systemic sensitization in the presence of an adjuvant. Therefore, we first characterized peanut-specific in C3H, CBA, BALB/c and SJL mice after peanut sensitization by ip injection in the presence of alum. This protocol of sensitization promoted strain-dependant IgG, IgG2a, IgG1 and IgE response (Figs. 2 and 3). All the strains presented a peanut-specific IgG response that cross-reacted with lupine (Table 1). The

Table 1
In vitro reactivity to peanut and lupine of ip peanut-sensitized mice IgG2a and IgG1 Ab

		Strain	Absorbance			
			Day 0	Day 15	Day 30	Day 45
IgG2a	Peanut (Dilution 1/10 ⁵)	C3H	0.11 ± 0.04	1.44 ± 1.00*	1.29 ± 0.47*	1.41 ± 0.49*
		CBA	0.19 ± 0.10	0.65 ± 0.37*	0.74 ± 0.46*	0.73 ± 0.64*
		BALB/C	0.14 ± 0.04	0.78 ± 0.62*	1.39 ± 0.29*	1.86 ± 0.83*
		SJL	0.100 ± 0.007	0.76 ± 0.50*	0.55 ± 0.37*	0.62 ± 0.24*
	Lupine (Dilution 1/400)	C3H	0.110 ± 0.006	0.11 ± 0.01	0.32 ± 0.25*	0.07 ± 0.53*
		CBA	0.11 ± 0.08	0.11 ± 0.01	0.23 ± 0.20	0.20 ± 0.16
		BALB/C	0.11 ± 0.006	0.18 ± 0.24	0.28 ± 0.38	0.40 ± 0.51
		SJL	0.110 ± 0.03	0.14 ± 0.12	0.38 ± 0.59	0.77 ± 0.70*
IgG1	Peanut (Dilution 1/10 ⁵)	C3H	0.43 ± 0.22	2.07 ± 0.36*	1.82 ± 0.29*	1.85 ± 0.26*
		CBA	0.12 ± 0.06	1.93 ± 0.56*	2.14 ± 0.10*	1.89 ± 0.26*
		BALB/C	0.21 ± 0.10	1.83 ± 0.40*	2.00 ± 0.18*	1.81 ± 0.10*
		SJL	0.10 ± 0.01	1.79 ± 0.48*	1.73 ± 0.40*	1.66 ± 0.47*
	Lupine (Dilution 1/400)	C3H	0.13 ± 0.06	0.46 ± 0.19	1.22 ± 0.14*	2.13 ± 0.34*
		CBA	0.11 ± 0.01	0.17 ± 0.10	0.71 ± 0.50*	1.06 ± 0.84*
		BALB/C	0.10 ± 0.01	0.12 ± 0.01*	0.36 ± 0.33*	0.65 ± 0.43*
		SJL	0.11 ± 0.01	0.48 ± 0.48*	1.07 ± 0.57*	1.61 ± 0.69*
IgG1/IgG2a	Peanut	C3H	3.81 ± 2 E ⁻³	1.43 ± 9 E ⁻³	1.40 ± E ⁻³	1.31 ± 3.8 E ⁻³
		CBA	0.61 ± 2 E ⁻⁴	2.95 ± 2 E ⁻³	2.89 ± 2 E ³	2.54 ± 3 E ⁻³
		BALB/C	1.47 ± 8 E ⁻⁴	2.36 ± 8 E ⁻³	1.44 ± 7 E ⁻⁴	0.97 ± 1.5 E ⁻³
		SJL	0.93 ± 1 E ⁻⁵	2.33 ± E ⁻³	3.10 ± E ⁻³	2.69 ± 1.3 E ⁻³
	Lupine	C3H	1.17 ± 6E ⁻⁴	2.05 ± 1.62 E ⁻³	3.76 ± 4.5 E ⁻³	3.10 ± 3.4 E ⁻³
		CBA	1.00 ± 5 E ⁻⁴	1.53 ± 1 E ⁻⁴	3.00 ± 4.6 E ⁻³	5.20 ± 5.8 E ⁻³
		BALB/C	0.93 ± 1 E ⁻⁴	0.65 ± 3.9 E ⁻⁴	1.27 ± 2.25 E ⁻³	1.62 ± 3.3 E ⁻³
		SJL	0.96 ± 1.4 E ⁻⁴	3.26 ± 2.4 E ⁻³	2.81 ± 5.4 E ⁻³	2.09 ± 4.2 E ⁻³

* Significantly different from day 0 ($p < 0.05$).

Table 2

Peanut specific antibodies in mice treated orally with peanut

	Strain	Absorbance				
		Day 0	Day 15	Day 30	Day 45	Day 60
IgG (Dilution 1/50)	C3H	0.35 ± 0.41	0.35 ± 0.35	0.30 ± 0.17	0.20 ± 0.08	0.18 ± 0.08
	CBA	0.14 ± 0.01	0.23 ± 0.06*	0.45 ± 0.78	0.23 ± 0.14*	0.18 ± 0.05*
	SJL	0.52 ± 0.39	0.52 ± 0.21	0.46 ± 0.21	0.55 ± 0.28	0.62 ± 0.40
IgG2a (Dilution 1/50)	C3H	0.22 ± 0.22	0.58 ± 0.12*	0.49 ± 0.23*	0.38 ± 0.15	0.35 ± 0.13
	CBA	0.12 ± 0.02	0.35 ± 0.10*	0.48 ± 0.60	0.32 ± 0.24*	0.25 ± 0.10*
	SJL	0.09 ± 0.02	0.16 ± 0.05*	0.14 ± 0.04*	0.22 ± 0.23*	0.21 ± 0.22
IgG1 (Dilution 1/50)	C3H	0.31 ± 0.34	0.39 ± 0.42	0.29 ± 0.24	0.25 ± 0.24	0.17 ± 0.11
	CBA	0.11 ± 0.02	0.12 ± 0.06	0.37 ± 0.73	0.14 ± 0.08	0.16 ± 0.08*
	SJL	0.61 ± 0.71	0.89 ± 0.64	0.55 ± 0.66	0.46 ± 0.62	0.52 ± 0.71
IgE (Dilution 1/50)	C3H	0.09 ± 0.02	0.09 ± 0.01	0.080 ± 0.005	0.080 ± 0.005	0.08 ± 0.004
	CBA	0.09 ± 0.03	0.080 ± 0.006	0.080 ± 0.005	0.080 ± 0.003	0.080 ± 0.003
	SJL	0.090 ± 0.007	0.090 ± 0.005	0.07 ± 0.01	0.080 ± 0.004	0.080 ± 0.005

* Significantly different from day 0 ($p < 0.05$).

IgG response was lower with lupine than with peanut and the lupine IgG response was in the order C3H>SJL>CBA>BALB/c. Peanut-specific IgE response was markedly higher in CBA than in BALB/c and C3H mice and a weak IgE response was observed in SJL mice. CBA, BALB/c and C3H peanut-sensitized mice also showed a weak lupine IgE cross-reactivity whereas no response was detected for SJL mice. The peanut-lupine IgE cross-reactivity in the three strains of mice was in the following order CBA>C3H>BALB/c (Table 1). The peanut and lupine IgG1/IgG2a response ratio were positive when peanut and lupine IgE responses were increased, with the exception of SJL mice for which the IgG1/IgG2a ratio was also positive, even in the absence of peanut IgE responses.

3.2. Different mouse strains display similar patterns of peanut-specific antibody responses to repeated oral peanut doses

Sensitization to food antigens generally occur by ingestion. This led us to address whether the MHC haplotype and other genetic factors also affected sensitization to peanut by the oral route. More specifically, we examined the levels of peanut-specific Abs responses in the serum of C3H, CBA and SJL mice that received oral peanut doses without adjuvant for three months. Our results indicated no difference between the mice strains which all exhibited very low level of peanut-specific IgG2a responses and no IgG1

Abs (Table 2). None of the mouse strains showed peanut-specific serum IgE responses after this oral sensitization (Table 2). Thus, the lack of IgE and presence of IgG2a but not IgG1 Abs suggested that a weekly oral sensitization to peanut could promote Th1 type responses.

3.3. Hypersensitivity response to peanut and lupine of mouse strains sensitized to peanut

The biological significance of peanut sensitization in different mouse strains was further evaluated by measurement of the response to ip challenge with peanut or lupine (Table 3). The peanut challenge produced clinical signs of allergic reaction (tremor, prostration) in peanut-sensitized C3H and to a lesser extent CBA mice. On the other hand, BALB/c and

Table 3

Hypersensitivity response in control and peanut-sensitized mice after ip challenge with peanut or lupine

Antigen	Strains	Scoring system
Peanut	C3H control	0
	C3H peanut-sensitized	5 (80%)
	CBA peanut-sensitized	4 (20%)
	BALB/c peanut-sensitized	0
	SJL peanut-sensitized	0
Lupine	C3H control	0
	C3H peanut-sensitized	0
	CBA peanut-sensitized	0
	BALB/c peanut-sensitized	0
	SJL peanut-sensitized	0

SJL mice did not show any clinical sign of allergy. Interestingly, none of the mouse strains showed signs of allergic reaction after ip challenge with lupine. No anaphylactic reaction was observed following peanut and lupine injection in mice immunized orally.

4. Discussion

It is now accepted that a number of parameters including the genetic background, the general immune status and the mode of sensitization contribute to food allergies. However, appropriate murine models are greatly needed to improve our understanding of mechanisms underlying these adverse immune reactions in humans. Recent studies have shown that susceptibility of mouse strains to peanut allergy was linked to differential T helper cell responses in different genetic backgrounds [19]. Cross-reactivity of peanut-specific antibody (Ab) with other legumes is frequently demonstrated but it still remains to be demonstrated whether these responses could lead to clinical signs of cross-allergenicity. Here we show that C3H, BALB/c, and CBA develop high levels of peanut-specific serum IgE, IgG1 Ab responses following intraperitoneal sensitization with peanut and alum as adjuvant. In addition, cross-allergenicity may not directly correlate with the presence of cross-reactive Abs since mice sensitized to peanut reacted with lupine in ELISA but failed to exhibit clinical symptoms of cross-allergenicity after challenge with lupine.

The most common immune response to ingested antigens is tolerance or systemic unresponsiveness. In order to overcome this tendency of orally administered antigens to generate tolerance, animal models of food allergies are being developed by oral co-administration of cholera toxin, a potent mucosal adjuvant that has been shown to enhance Th2-type response with IL-4 and IgE Abs in some mouse strains [21,22]. In this regard, intragastric gavage of mice with peanut protein and cholera toxin as adjuvant was shown to generate peanut-specific serum IgE Abs and hypersensitivity [18]. Other studies have shown that feeding a protein antigen as a constituent of the diet in a natural way without adjuvant can induce Th2-like response with high levels of systemic IgE in mouse [23] and rat strains (BN rats) [24]. We

have shown that a weekly oral dose of peanut protein as part of the normal diet do not promote peanut IgE-specific Abs in C3H, CBA and SJL mice. The weekly oral peanut dose only induced IgG2a Abs and failed to set the stage for systemic anaphylactic symptoms. Our findings confirm the immunogenicity of peanut proteins. They also suggest that, in contrast with oral sensitization with peanut and CT as adjuvant which was shown to promote different T helper cell responses in C3H/HeJ and BALB/c mice [19], peanut proteins given without adjuvant as a constituent of the diet, peanut proteins could promote Th1-type responses in all three mouse strains. However, more studies will be needed to clearly demonstrate the exact pattern of Th cell cytokine responses promoted by oral sensitization with peanut alone.

In contrast with oral sensitization in the absence of adjuvant, the mice strains exhibited different responses after intraperitoneal sensitization. Thus, all the four strains developed peanut-specific IgG whereas only three strains (C3H, CBA, BALB/c) presented peanut-specific IgE Abs. In addition, only two out of the three strains that developed IgE (C3H, CBA) exhibited symptoms of allergy upon peanut challenge. In our studies, anaphylactic reactions were seen after peanut challenge of C3H (H-2^k) and CBA (H-2^k) mice but not in BALB/c (H-2^d) or SJL (H-2^s) mice. Anaphylactic responses were reported after peanut challenge of C3H (H-2^k) but not AKR (H-2^k) or BALB/c (H-2^d) mice previously sensitized by administration of a plasmid DNA encoding peanut [4]. Therefore, while a number of parameters influence the immune responses, the H-2^k phenotype appears to contribute to the development of allergic-type immune responses such as those seen in C3H mice.

It is now established that IgE Abs play an important role for type 1 hypersensitivity in human [25,26] and animal [27,28]. Other murine studies suggest that immediate hypersensitivity and airway hyperresponsiveness could be passively transferred by allergen-specific IgE and IgG1, but not IgG2a or IgG3 [18,29]. These studies are consistent with our finding that C3H and CBA mice which presented peanut-specific IgE and IgG1 Abs were the only strains that developed anaphylactic reactions upon peanut challenge. They are also consistent with the lack of anaphylactic reactions seen in SJL mice which exhibited peanut-specific IgG1 and IgG2a but not

IgE Abs. Interestingly, peanut-sensitized BALB/c mice produced peanut-specific IgE and IgG1 Abs but failed to develop clinical sign of allergy after peanut challenge. Thus, the induction of allergen-specific IgE and IgG1 Abs may not always lead to anaphylactic reactions in mouse models.

Antibody cross-reactivity generally reflects the phylogenetic relations that lead to proteins with high degree of primary structure homologies [30]. On the other hand, cross-allergenicity refers to allergic reactions elicited by antigen challenge after sensitization with a different antigen. Peanut-specific IgE Abs were shown to cross-react with homologous proteins in soybean and other legumes [30]. Nonetheless, only rare cases of cross-allergy between legumes have been reported [17]. In the present study, C3H, CBA and BALB/c mice sensitized to peanut contained IgE Abs which cross-reacted with lupine in vitro. The levels of cross-reactive IgE Abs were lower than those of peanut-specific IgE. More importantly, none of the mouse strain with peanut-specific IgE Abs showed signs of anaphylactic reaction after lupine challenge indicating that cross-allergic reaction did not occur.

In conclusion, several parameters including mice strains, the route of sensitization, the nature and dose of the antigens and the adjuvants should be considered in mouse models of food allergy. Our studies indicate that C3H mice represent a valuable model of peanut allergy capable of developing peanut-specific IgE and IgG1 Abs and anaphylactic responses upon peanut challenge. We also provided evidence suggesting that in vitro cross-reactivity of IgE and IgG1 Abs from peanut-sensitized mice with lupine flour may not translate into anaphylactic symptoms when mice are challenged with lupine. Taken together, our studies also confirm that appropriate animal models are needed in order to better delineate the mechanisms underlying primary food allergies and cross-allergenic reactions.

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