

A strong association between HLA-DR9 and gelatin allergy in the Japanese population

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Abstract

The frequency of HLA class I and II phenotypes was determined among 23 patients with positive gelatin IgE, eight of whom developed anaphylaxis, 18 patients who did not have gelatin IgE but who experienced non-immediate reactions after exposure to gelatin. HLA-DR9, which is unique to Orientals, was present in 56.5% of the gelatin IgE positive patients, as compared to control population frequency of 24% ($P < 0.002$). In the non-immediate reaction group, who did not generate IgE, phenotype distribution resembled controls. HLA-DR9 positive individuals have a relative risk of 4.1 for developing gelatin allergy with positive IgE. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Gelatin containing vaccines occasionally cause clinical reactions including anaphylaxis and gelatin specific IgE has been detected in sera obtained from patients with immediate reactions [1,2]. Furthermore, sensitized subjects develop gelatin specific lymphocyte responses [3]. Recent studies have focused on the pathogenesis of this particular allergy [4,5]. Epidemiologic studies in Japan suggest that gelatin containing diphtheria tetanus pertussis (DTP) vaccine is causally related to primary sensitization to gelatin [6,7]. Based on studies using a lymphocyte proliferation test (LPT) and an IL-2 responsiveness assay, we published the first direct laboratory-based evidence, that inoculation of gelatin

containing DTP vaccine causes sensitization to gelatin in the recipients [8]. These findings convinced us that subjects are primarily sensitized by gelatin in DTP vaccine and develop subsequent clinical reactions due to re-exposure to gelatin contained in medical preparations or in various foods.

However, it is unknown why only some subjects generate gelatin IgE, whereas so many subjects inoculated with gelatin containing DTP vaccine are sensitized [8]. It is important to identify subjects with established sensitization, who might generate gelatin specific IgE in the future, because the presence of IgE poses a considerable risk of anaphylaxis in case of re-exposure to gelatin. Because almost all reports of gelatin allergy are from Japan, an ethnic basis has been proposed [9]. In the present study, we determined HLA class I and II phenotypic frequencies in a population of subjects with gelatin allergy and analyzed the relationship between these phenotypes and the development of gelatin allergy with positive IgE specific to gelatin.

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2. Materials and methods

2.1. Subjects

The study groups were composed of 23 children (8 boys, 15 girls) with immediate type reactions, with a mean age of 1.8 yr (1.1–4.9 yr), and 18 children (11 boys, 7 girls) with non-immediate type reactions, with a mean age of 1.9 yr (1–4.9 yr), who were diagnosed as having a gelatin allergy from 1995 to 1997 (Table 1). The diagnosis of gelatin allergy depended on a positive gelatin specific IgE and/or a positive reaction in either

or both of a LPT and an IL-2 responsiveness assay specific to conventional gelatin. Adverse reactions were varied. Immediate reactions consisted of urticaria and anaphylaxis with respiratory and cardiovascular symptoms. Some children had sharply demarcated redness, swelling and induration at the injection site exceeding 1 cm in diameter, appearing more than 12 h after administration of vaccine and persisting for a few days. Another type of adverse reaction consisted of a measles-like miliary rash without itching also appearing more than 12 h after administration of vaccine and disappearing without any pigmentation within a couple

Table 1
Clinical and laboratory data on patients

Patient no.	Age (month)	Sex	Cause	Reactions	Gelatin IgE (Ua/ml)	LPT (SI)	AIR (ΔSI)
<i>Immediate</i>							
1	34	F	MMR	Anaphylaxis	46.90	1.90	0.13
2	59	F	Mumps	Anaphylaxis	30.70	2.40	0.49
3	15	F	Measles	Anaphylaxis	3.92	1.40	0.38
4	22	F	Measles	Anaphylaxis	2.20	5.20	0.07
5	27	F	Gummy	Anaphylaxis	2.20	2.58	0.31
6	24	F	Measles	Anaphylaxis	5.27	3.60	0.28
7	36	M	Rubella	Anaphylaxis	14.20	1.62	0.23
8	13	F	Measles	Anaphylaxis	5.26	3.12	0.17
9	14	F	Measles	Urticaria	5.95	3.90	0.12
10	13	F	Measles	Urticaria	1.62	2.40	0.21
11	20	F	Measles	Urticaria	1.86	NT ^a	NT
12	16	M	Measles	Urticaria	0.83	2.39	0.38
13	14	M	Measles	Urticaria	0.76	7.80	0.97
14	24	F	Measles	Urticaria	0.67	1.66	0.08
15	15	M	Measles	Urticaria	2.04	3.42	0.15
16	23	F	Measles	Urticaria	5.98	6.54	0.38
17	15	F	Measles	Urticaria	3.64	4.80	0.36
18	26	M	Mumps	Urticaria	10.00	2.00	0.06
19	13	M	Measles	Urticaria	3.02	1.00	0.78
20	15	F	Measles	Urticaria	0.65	1.59	0.28
21	17	F	Measles	Urticaria	0.50	5.20	0.58
22	26	M	Measles	Urticaria	6.32	1.70	0.50
23	16	M	Measles	Urticaria	0.96	NT	NT
<i>Non-immediate</i>							
1	33	F	Measles	Erythema, swelling	0.05 >	1.00	0.19
2	12	M	Measles	Erythema, swelling	0.05 >	10.50	0.72
3	36	F	Measles	Erythema, swelling	0.05 >	6.21	0.43
4	16	F	Measles	Miliary rash	0.05 >	1.42	0.33
5	14	F	Measles	Erythema, swelling	0.05 >	0.50	0.48
6	13	F	Measles	Erythema, swelling	0.05 >	1.03	0.27
7	18	F	Measles	Erythema, swelling	0.05 >	0.80	0.31
8	15	M	Measles	Miliary rash	0.05 >	0.80	0.35
9	15	M	Measles	Erythema, swelling	0.05 >	1.30	0.38
10	16	F	Measles	Erythema, swelling	0.05 >	2.20	0.56
11	14	M	Measles	Erythema, swelling	0.05 >	0.50	0.49
12	15	M	Measles	Erythema, swelling	0.05 >	6.23	0.40
13	22	M	Measles	Erythema, swelling	0.05 >	NT	0.78
14	22	M	Measles	Erythema, swelling	0.05 >	4.85	0.62
15	13	M	Measles	Erythema, swelling	0.05 >	7.35	0.09
16	36	M	Rubella	Erythema, swelling	0.05 >	NT	0.13
17	59	M	Rubella	Erythema, swelling	0.05 >	NT	0.55
18	48	M	Rubella	Erythema, swelling	0.05 >	NT	0.18

^a Not tested.

Table 2

Phenotype frequencies of the HLA antigens in Japanese patients with gelatin allergy with or without IgE specific to gelatin

Gelatin IgE	HLA	Patients (%) ^a	Controls (%) ^a	χ^2 ^b	<i>P</i> value	<i>P_c</i> value ^c	Relative risk
IgE (+)	B48	21.7 (5/23)	7.6 (24/317)	3.851	0.05	NS ^d	4.1
	DR9	56.5 (13/23)	24.0 (24/100)	9.403	0.002	0.034	
	DQ3	73.9 (17/23)	44.4 (32/72)	6.061	0.014	NS	
IgE (–)	A31	38.9 (7/18)	13.9 (44/317)	8.254	0.005	NS	
	DR8	38.9 (7/18)	17.0 (17/100)	4.511	0.042	NS	
	DR9	44.4 (8/18)	24.0 (24/100)	3.226	NS	NS	

^a Frequency of phenotypes carrying the allele.^b χ^2 test with Yates' correction if needed.^c Corrected for the number of alleles tested.^d Not significant.

of days. In subjects in the present study, immediate type reactions were elicited by inoculation of gelatin containing vaccines in 22 cases and by eating food-containing gelatin in one case, while non-immediate type reactions were elicited by gelatin containing vaccines in all cases. Informed consent was obtained from the parents of the patients after the project was thoroughly explained.

2.2. Measurements of gelatin specific IgE

IgE antibody to bovine gelatin (Wako Junyaku, Tokyo, Japan) was measured by a fluorometric ELISA system previously described [8]. The positive/negative cut-off was set at 0.05 Ua/ml.

2.3. Gelatin specific lymphocyte responses

The in vitro LPT used resembles that described previously [3,8]. A stimulation index ≥ 1.5 was considered to be a positive response. Antigen specific interleukin 2 responsiveness (AIR) has also been described previously [3]. An SI increment (Δ SI) of 0.08 or more was regarded as a positive response.

2.4. HLA class I and II typing

HLA class I (HLA-A, B and C) antigens were determined by a standard microlymphocytotoxicity test. Well-characterized antisera obtained commercially and through serum exchange among the Japanese Red Cross Blood Centers were used to identify 32 HLA class I antigens. HLA class II (HLA-DR, DQ) typing was performed by DNA-based techniques (low resolution) of allele specific oligonucleotide hybridization (DRB1) or restriction fragment length polymorphism (DQB1) after amplification of corresponding DNA. Typing results were translated into 17 serological phenotypes. Control typing of HLA antigens was performed in 72 to 317 healthy, unrelated employees of the Hokkaido Red Cross Blood Center from the same

region. The frequencies of HLA antigens in patients and controls were compared using the χ^2 test, with Yates' correction if needed, with 2×2 contingency tables. The corrected *P* (*P_c*) values were obtained by multiplying *P* values by the number of alleles tested. The level of significance was set at 0.05. Relative risk was calculated as the cross product in a 2×2 table as reported elsewhere [10].

3. Results

3.1. Patients

The clinical and laboratory features of subjects included in the study are summarized in Table 1. The interval between clinical reactions and blood sampling varied in each case. All subjects with immediate type reactions developed positive gelatin specific IgE with a range from 0.50 to 46.90 Ua/ml. Nineteen out of 21 subjects with immediate reactions tested positive both for LPT and AIR. None of the subjects with non-immediate reactions had gelatin specific IgE, although the LPT in 6 out of 14 cases tested and the AIR in all 18 cases were positive.

3.2. HLA typing

Specificity for HLA-DR9 was present in 56.5% of the gelatin IgE positive patients, compared to control population frequencies of 24% ($P < 0.002$) (Table 2). HLA-DR9 positive individuals have a 4.1-fold risk of developing gelatin allergy with positive IgE.

P values of B48, DQ3 in a group of patients with IgE positive gelatin allergy and HLA-A31, DR8 in a group of patients with IgE negative gelatin allergy were significant, however, when *P* values were corrected for the number of alleles tested (*P_c*), only HLA-DR9 was shown to be associated with gelatin allergy. No unusually frequent phenotypes were observed in patients who did not generate IgE to gelatin.

4. Discussion

The main purpose of the present study was to determine whether there was any association between HLA phenotypes and the specific IgE response against gelatin. We have shown that HLA-DR9 is strongly associated with the development of IgE positive gelatin allergy in Japan. HLA-DR9 is widely distributed in Japanese but not in Caucasians [11], suggesting that the highly concentrated prevalence of gelatin allergy in Japan can be explained by the results of the present study. Genetic susceptibility to gelatin allergy that is related to the immune responsive gene is assumed to be closely related to HLA-DR9. It is surprising that analysis of a relatively small number of patients with positive IgE revealed such a strong association with a single specific HLA. DR9 may play a crucial role in exposing the immunodominant regions of processed gelatin antigens to helper T cells.

Of alleles other than DR9, DQ3 is known to be a strong linkage disequilibrium with allele DR9 in the Japanese population. Thus, it is understandable that DQ3 revealed a relatively high χ^2 value with a low *P* value, although it is no longer significant with a corrected *P* value. It can be assumed that DQ3 is not independently associated with gelatin allergy and that only the DR9 allele is directly involved in the susceptibility to gelatin allergy with positive IgE.

The present study also showed no unusual frequent phenotypes in patients with non-immediate reactions who did not generate IgE to gelatin. This finding is not surprising because anyone could be sensitized to gelatin at the lymphocyte level by "optimal" stimulation such as gelatin injected with alum adjuvant [8]. We believe that subjects with non-immediate reactions belong to an immunogenetically different population from those with immediate reactions.

In summary, our results seem to provide a key answer to the question why there are so many reports of gelatin allergy in Japan compared to other countries. Our results should be of great help in identifying T cell epitopes of gelatin as allergen and in developing possibly non-allergenic gelatin in the future. Detailed analysis of HLA-DR9 in relationship to gelatin allergy such as was done for birch pollen allergy will be necessary [12]. Further studies are still necessary to elucidate why the number of patients with gelatin allergy has dramatically increased during recent years despite the same genetic background as substantiated by the distribution of HLA-DR9 in Japan. Fundamental societal changes in Japan, including dietary habits, might be related and studies of this issue are in progress.

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