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Analysis of HLA in children with gelatin allergy

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anaphylaxis; gelatin; HLA; IgE; vaccine

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Abstract: Systemic immediate reactions including anaphylaxis to gelatin in vaccines have been reported. However, the number of such reports is very small compared with the number of children exposed to gelatin. The present study was designed to investigate whether susceptibility or resistance to gelatin allergy is associated with human leukocyte antigen (HLA) class II gene. Blood samples were obtained from 49 patients with gelatin allergy and specific IgE to gelatin. DNA-based HLA class II typing was performed to determine the DRB1, DQB1 and DPB1 alleles. Genotype frequencies were compared with those found in 240 unrelated controls. The frequency of DQB1*0303 (55.1%) was significantly higher in the patients than in the control subjects (32.1%). The frequency of DPB1*0402 was also significantly higher in the patients (32.7%) than in the control subjects (15.4%). On the other hand, the frequency of subjects carrying DRB1*15 (DRB1*1501 and DRB1*1502) was significantly lower among the patients group (18.4%) than among the controls (40.8%). We found that DQB1*0303 and DPB1*0402 were positively associated with the IgE response for gelatin, while DRB1*15 was negatively associated with it.

Anaphylactic reactions to measles, mumps, and rubella vaccines and to the combined measles-mumps-rubella (MMR) vaccine have been reported, and it has been suggested that they are caused by an allergy to the egg proteins present in the vaccines (1). However, anaphylactic reactions have also been reported after the administration of the MMR vaccine to children who tolerate egg (2).

Kelso et al. reported that a child who suffered from anaphylaxis to the MMR vaccine had the IgE antibody to gelatin (3). Also, in previous studies (4–6), we found that most of the children who had the systemic immediate-type reactions, including anaphylaxis, to measles, mumps, and rubella vaccines had antigelatin IgE. In a recent epidemiological study, approximately 90% of children who showed the allergic reactions to vaccines had antigelatin IgE (7). This finding suggested that most of the systemic immediate-type reactions after vaccination are caused by the gelatin that is included

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in the live vaccines as a stabilizer. Furthermore, we found that the systemic reactions to varicella and Japanese encephalitis vaccines containing gelatin are also caused by the gelatin in the vaccines (8, 9). It has been reported that gelatin causes food allergy (5). Recently, we found that anaphylaxis to erythropoietin products containing gelatin was caused by gelatin (10).

The incidence of anaphylaxis to MMR vaccines in the US was reported to be 0.5 cases per million doses (11). In Japan, the estimates for the incidence of severe anaphylaxis in 1994–96 were 6.84, 7.31, 4.36, and 10.3 cases per million doses of gelatin-containing measles, rubella, mumps, and varicella vaccines, respectively (7). It seems that the number of patients with gelatin allergy is very low.

However, the mechanism by which allergic children are sensitized to gelatin is unclear. In Japan, DTaP vaccines either with or without gelatin were available (12). Previously, we explored the possibility that the administration of gelatin-containing DTaP vaccines before live viral vaccines sensitized children to gelatin. We found that there was a strong causal relationship between gelatin-containing DTaP vaccination, antigelatin IgE production, and the risk of anaphylaxis following subsequent immunization with live viral vaccines that contain larger amounts of gelatin (13, 14). Kumagai et al. recently reported an association of DR9 with the IgE-positive gelatin allergy by analyzing HLA-DR and DQ in 23 patients (15). However, no report has analyzed the relationship between the immune response to gelatin and HLA-DP.

To investigate the relationship between the immune response to gelatin and HLA in more detail, we analyzed HLA-DR, DQ and DP in 49 children who showed immediate reactions to a gelatin-containing vaccine and/or to food and had antigelatin IgE.

From physicians throughout Japan, we received blood samples from 49 children who experienced systemic immediate reactions to gelatin-containing vaccines and/or food. The data on the children were obtained by direct physician contact. Of the 49 children, 35 showed systemic immediate reactions to gelatin-containing vaccines, six showed immediate reactions to both gelatin-containing vaccines and food, and eight showed immediate reactions to gelatin-containing food only. Of the 41 children who showed a systemic allergic reaction to vaccines, nine had suffered from severe anaphylaxis including cutaneous signs (e.g., systemic urticaria, angioedema) plus airway obstruction (with laryngeal edema or wheezing) or anaphylactic shock (with hypotension and vascular collapse), 17 suffered from mild anaphylaxis (e.g., systemic urticaria and/or wheezing and/or cough) without severe symptoms, and 15 displayed only systemic cutaneous signs. These systemic reactions appeared within 1 h of vaccination. Of the 14 children who showed allergic reactions to gelatin contained in food, four had suffered from severe anaphylaxis including cutaneous signs (e.g., systemic urticaria, angioedema)

plus airway obstruction (with laryngeal edema or wheezing) or anaphylactic shock (with hypotension and vascular collapse), three suffered from mild anaphylaxis (e.g., systemic urticaria and/or wheezing and/or cough) without severe symptoms, and seven displayed only systemic cutaneous signs. These systemic reactions appeared within 1 h of exposure to gelatin in food. As a control, 240 healthy unrelated volunteers selected at random were analyzed for the HLA polymorphism. They were chosen regardless of atopic diseases.

Blood collection for the measurement of IgE and analysis of HLA was carried out within 1 month of the adverse reactions. The CAP system (Pharmacia, Uppsala, Sweden) was used to determine the concentration (Ua (Unit allergen)/mL) of IgE to bovine gelatin (Wako Pure Chemical Industries, Osaka, Japan) at the National Institute of Infectious Diseases. Genomic DNA was extracted from the peripheral blood leukocytes. HLA class II genotypes (DRB1, DQB1, DPB1) were determined by the polymerase chain reaction (PCR) in combination with a sequence-specific oligonucleotide probe (SSOP) as described previously (16). The odds ratio (OR) of the disease was calculated according to Wolf's or Haldane's formula. The statistical significance was examined by a chi square (χ^2) test with Yates' correction. The corrected *P*-value (*P_c*) was calculated by multiplying the *P*-value (*P*) by the number of alleles tested for each locus. A two-locus analysis was conducted according to the principle described by Svejgaard et al. (17).

Forty-nine children (male 24, female 25; mean age $\pm$ SD, 2 years, 4 months $\pm$ 1 year, 1 month) were studied with antigelatin IgE ranging from 1.1 to 192 Ua/mL (geometric mean value 11.0 Ua/mL). Of the 49 children, 35 with immediate reactions to gelatin-containing vaccines had antigelatin IgE ranging from 1.9 to 192 Ua/mL (geometric mean 13.5 Ua/mL), six, with immediate reactions to both gelatin-containing vaccines and food, had antigelatin IgE ranging from 3.5 to 14.0 Ua/mL (geometric mean value 9.2 Ua/mL), and eight with immediate reactions to only gelatin-containing food had antigelatin IgE ranging from 1.1 to 37.0 Ua/mL (geometric mean value 5.3 Ua/mL). There was no significant difference in the antigelatin IgE levels among these groups.

The distribution of DR alleles among the patients and the control subjects is shown in Table 1. The frequency of subjects carrying HLA-DRB1*15 (DRB1*1501 and DRB1*1502) was significantly lower among the patient group (18.4%) than among the controls (40.8%) (*P_c* = 0.048). The frequency of DRB1*0901 was higher in the patient population (48.9% vs 31.3%, OR = 2.11), but the difference lost significance after *P*-value correction (*P* = 0.017, *P_c* = 0.28) (data not shown). There was no significant difference between the two groups in the distribution of the other DR alleles (data not shown). Negative associations between HLA-DR alleles and allergic diseases have been reported such as the association between DR4

Distribution of HLA-DRB1, DQB1, and DPB1 alleles among patients and control groups

HLA	Patient group (n = 49)		Control group (n = 240)		Statistical tests		
	n	%	n	%	OR	P	P _c
DRB1*15 ^a	9	18.4	98	40.8	0.33	0.0030	0.048
DQB1*0303	27	55.1	77	32.1	2.60	0.0022	0.024
DPB1*0402	16	32.7	37	15.4	2.66	0.0045	0.036

^aDRB1*15 includes DRB1*1501 and DRB1*1502**Table 1**

and mountain cedar pollinosis (18) and that between DR4 and bee venom allergy (19). Although the immunological mechanism of the negative associations between DR and allergic diseases has not been defined, HLA-DR molecules may exert a protective effect against certain antigens. The molecular mechanism of the HLA-DR involvement in protecting subjects from gelatin allergy awaits further investigation.

There have been many studies showing correlation between HLA-DR and many allergic diseases, such as the association between DR2 and ragweed (20), DR5 and ryegrass (21), DRB1*01 and cockroach (22), and DRB1*1101 and house dust mite (23). However, we failed to find a significant association between HLA-DR allele and gelatin allergy. To date, the strongest and most consistent association is with HLA-DR2 and Amb a 5 of the ragweed pollen allergen, which consists of a small peptide of 45 amino acids (20). In general, large protein antigens express multiple T cell epitopes, while small polypeptides may express only a very small number of T cell epitopes that can be presented to T cells in association with particular HLA class II alleles. Large proteins may make associations between HLA class II alleles and allergic diseases less clear in the allergic patient population.

The distribution of DQ alleles among the patients and the control subjects is shown in Table 1. A significantly higher frequency of DQB1*0303 was apparent in the patients (55.1%) than in the control subjects (32.1%) ($P_c = 0.024$). There was no significant difference between the two groups in the distribution of other DQ alleles (data not shown). Several relationships between HLA-DQB1 and allergic diseases have been reported (24–26). For example, Kumai et al. reported that the HLA-DQ3 phenotype was strongly associated with the development of birch pollen allergy in the Japanese population (27). It also has been shown that the epitope on a birch pollen allergen can be presented by the HLA-DQ molecule (28). O'Brien et al. reported that T cell proliferative responses to a major mite allergen were positively associated with DQ7 (29). In gelatin allergy, DQB1*0303 might be considered to be a susceptibility factor for IgE production against gelatin.

As shown in Table 1, the frequency of the allele DPB1*0402 was significantly higher in the patients (32.7%) than in the control subjects (15.4%) ($P_c = 0.036$). There was no significant difference between the two groups in the distribution of other DP alleles (data not shown). Although the function of HLA-DP has not been fully elucidated, it has been reported that HLA-DP can present foreign and self peptides to activated T cells (30–32). Furthermore, several allergic diseases such as asthma, pollinosis and peanut allergy were associated with specific DP alleles (33–36). In Japan, the frequency of HLA-DP5 was significantly increased in patients with Japanese cedar pollinosis (34). O'Hehir et al. identified a T-cell clone for which the HLA DP*0401 allele restricts recognition of house dust mite allergen (37). These observations suggest that HLA-DP alleles might not be merely a genetic marker but may also be involved in the pathogenesis of the allergic diseases.

We investigated the relationship between alleles in term of the risk of gelatin allergy. The basic data for this analysis are shown in Table 2A, and the various two-by-two comparisons that were performed are shown in Table 2B. DQB1*0303 significantly increased the risk in the DPB1*0402-negative population (test 1, odds ratio = 3.25, $P = 0.0017$). DPB1*0402 increased the risk in the DQB1*0303-negative population (test 2, odds ratio = 3.55, $P = 0.0050$) and in the DQB1*0303-positive population (test 4, odds ratio = 3.38, $P = 0.043$). The presence of both disease-associated genetic markers showed a higher risk (test 6, odds ratio = 11.00, $P = 0.000014$) than did the presence of only one marker (tests 1 and 2). There was no linkage disequilibrium between DQB1*0303 and DPB1*0402 in Japanese populations (data not shown). In this study, the analysis suggested that DQB1*0303 and DPB1*0402 were independent risk factors. The two-locus analysis also demonstrated that individuals having both markers showed a higher risk for the allergy than those having only

Two-locus analysis of genetic risk to gelatin allergy

A) Basic data			
DQB1*0303	DPB1*0402	Patients	Control
+	+	6	6
+	–	21	71
–	+	10	31
–	–	12	132
B) Two-by-two comparison			
Test	Comparison	Odds	P
1	++ vs --	3.25	0.0017
2	-- vs --	3.55	0.0050
3	++ vs ++	3.10	ns (0.089)*
4	++ vs +-	3.38	0.043
5	+- vs ++	0.92	ns (0.84)*
6	++ vs --	11.00	0.000014

*ns; not significant ($P > 0.05$)**Table 2**

one marker, suggesting that DQB1*0303-linked factor and DPB1*0402-linked factor may confer synergistically the genetic risk.

Kumagai et al. recently reported the association between DR9 with the IgE-positive gelatin allergy in analysis of HLA-DR and DQ in 23 patients (15), during the preparation of this manuscript. We also found the increased frequency of DRB1*0901 in the patient group, consistent with the report by Kumagai et al. although in our case it was not statistically significant after correction of the *P*-value. Instead we revealed the significant association with DQB1*0303 that is in strong linkage disequilibrium with DRB1*0901 in Japanese popu-

lations. Because the OR conferred by DQB1*0303 was higher than that conferred by DRB1*0901 (2.60 vs 2.11), it was speculated that the DQB1*0303 was primarily associated with the IgE-positive gelatin allergy, and that the increased frequency of DRB1*0901 in the patients may merely reflect the linkage disequilibrium of DRB1*0901 with DQB1*0303. This point should be testable in the cases studied by Kumagai et al. because they noted that the DQ alleles were typed at the DNA level. However, detailed data were not presented by Kumagai et al. they instead presented the frequencies of serologically equivalent DQB1 alleles.

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