J. Tokyo Med. Coll., **54**(2): 160～167, 1996

Association between Wheat Allergy and HLA

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Abstract

Allergic responses are determined not only by environmental exposure but also genetic factors including the HLA gene which present antigens to T cells. Marked HLA polymorphism may contribute to the development of allergic symptoms. We studied wheat flour allergy symptoms and immunological factors such as wheat flour RAST in 15 bakers and investigated the relation between wheat flour RAST positivity and HLA serological types and allele sequences. The results were statistically compared to those of the general population in Japan, as reported at The 11th International Histocompatibility Workshop and Conference, as a control.

In the serological study, the frequencies of the Aw33 (w19), B44 (12) and Bw67 serotypes were significantly higher in wheat flour RAST-positive cases. No significant differences were observed between the study sugbjects and controls in the prevalence HLA-DR and HLA-DQ. In 10 of our subjects, genotyping of HLA class II alleles carried out by the PCR-RFLP method showed that the frequencies of DQB1＊0604 and DRB1＊1302 were significantly higher than controls. DPB1＊0401, DQB1＊0303, DQB1＊0602 and DRB1＊1502 were also increased, but the differences did not reach statistical significance. In haplotype frequency, 4 of 10 workers were found to be DRB1＊1302-DQB1＊0604-DPB1＊0201 haplotype, followed by 3 of 10 workers who were DRB1＊1502-DQB1＊0601-DPB1＊0501 haplotype. These data suggested that some types of HLA might play important roles in atopic mechanisms.

Introduction

Individual responses to certain antigens very widely, and studies of atopic disease have provided new insights into the genetic mechanisms underlying human immune responses. It is clear, however, that atopic responses are specific to certain antigens, and it is generally accepted that a relation exists between allergic symptoms and HLA (human leukocyte antigen) alleles. The polymorphic MHC class II molecules present on cell surfaces play a key role in T-cell activation, leading Marsh et al to postulate that each subject has an "allergic finger print"[1].Furthermore, allergies related to or stemming from a specific occupation provide an excellent opportunity to examine the immunologic mechanisms underlying atopic phenomena. It has been recognized that bakers, who work in an atmosphere permeated with wheat flour and grain products, frequently suffer from allergic disorders, such as nasal discharge, skin eruption,

Received Dec. 26, 1995, Accepted Feb. 9, 1996
Key words : Wheat flour allergy, HLA, Occupational allergy.
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conjunctival itching and coughing, a condition which is sometime referred to as "baker's asthma"[2]. Such responses are determined by both genetic factors and environmental exposure. In this study, we investigated the relation between wheat flour RAST (radioallergosorbant test) positivity and HLA by determining serological types and sequencing the alleles of subjects allergic to wheat flour.

Patients and Methods

The study population consisted of 15 wheat flour-specific IgE-positive males (average age : 27.7 years, SD : 6.95) who handled wheat flour daily while baking bread or making confectionery (Table 1).

Data concerning the duration of exposure (employment) and clinical symptoms were obtained by a questionnaire. Allergic status was evaluated by serum IgE (U/ml) and wheat flour-specific IgE (PRU/ml). In these 15 cases, serologically defined HLA types were examined by the Terasaki-NIH-Standard method[3]. Furthermore, in 10 cases, genotyping of HLA class II alleles was carried out by the SMITEST HLA DNA typing system based on the PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method[4].

Each value was statistically compared with the frequencies of the general population in Japan, as reported at The 11th International Histocompatibility Workshop and Conference[5], as a control. We used the relative risk and δ value to evalulate the relation between HLA haplotypes and wheat flour allergy. We also analyzed the data using by the χ^2 test and Fisher's exact test[6]. The frequencies of HLA serotypes are expressed as

Table 1. Worker Profiles

Case (NO.)	Age (y.o.)	Exposure (year)	Total IgE (IU/ml)	Wheat RAST (PRU/ml)	Nasal sympton	Cutaneous symptom	Conjunctival symptom	Respiratory symptons
NO. 1	36	10	380	17.5 ↑	+	−	−	+
NO. 2	28	4	670	17.5 ↑	+	−	−	+
NO. 3	37	15	590	17.5 ↑	−	+	+	+
NO. 4	38	10	245	10.5	−	−	+	+
NO. 5	23	4	160	8.58	+	+	+	−
NO. 6	22	22	460	8.48	+	−	−	−
NO. 7	20	0.17	2400	7.5	+	+	−	+
NO. 8	25	5	410	5.4	−	−	−	−
NO. 9	22	3.5	100	5.02	+	+	−	+
NO.10	29	12	56	4.93	+	+	+	+
NO.11	23	5	280	4.8	+	−	−	+
NO.12	23	4	8000	2.87	+	−	+	−
NO.13	30	3	83	2.77	−	−	−	−
NO.14	20	0.16	5400	1.92	−	−	−	−
NO.15	40	16	85	1.02	+	−	−	−

Table 2. Serological HLA types in wheat allergy

Antigen HLA	Workers (n=15)		Normal Japanese (n=1023)		Relative risk	δ	Chi square	p Value	Fisher one tall
	number	gene frequency (%)	number	gene frequency (%)					
A1	1	6.7	7	0.7	10.37	0.06	1.307	0.253	0.1103
A2	7	46.7	250	24.4	2.71	0.294	2.819	0.0932	0.0663
A3	1	6.7	6	0.6	12.11	0.06	1.606	0.205	0.0971
A11	2	13.3	106	10.4	1.33	0.033	0	1	0.4735
A24(9)	8	53.3	359	35.1	2.11	0.281	1.428	0.2321	0.1746
A26(10)	1	6.7	112	10.9	0.58	−0.048	0.012	0.9116	0.5014
A31(19)	2	13.3	82	8	1.77	0.058	0.074	0.785	0.3459
Aw33(w19)	6	40	79	7.7	7.97	0.35	16.418	<0.0001	0.0007
B7	2	13.3	51	5	2.93	0.088	0.752	0.3857	0.1759
B15	1	6.7							
B17	1	6.7							
B35	2	13.3	83	8.1	1.74	0.057	0.066	0.7966	0.3514
B37	1	6.7	7	0.7	10.37	0.06	1.307	0.253	0.1103
B44(12)	5	33.3	76	7.4	6.23	0.28	10.243	0.0012	0.0041
Bw4B	1	13.3	33	3.2	4.62	0.104	2.052	0.152	0.0881
B51(5)	3	20	95	9.3	2.44	0.118	0.929	0.335	0.1617
Bw52(5)	4	26.7	109	10.7	3.05	0.179	2.431	0.119	0.0705
Bw54(w22)	1	6.7	64	6.3	1.07	0.004	0	1	0.6235
Bw55(w22)	1	6.7	30	2.9	2.364	0.036	0.006	0.9366	0.3674
Bw60(40)	2	13.3	57	5.6	2.61	0.082	0.529	0.4871	0.208
Bw61(40)	1	6.7	109	10.7	0.6	−0.045	0.006	0.9397	0.5164
Bw62(15)	1	6.7	85	7.3	0.79	−0.018	0	1	0.6431
Bw67	2	13.3	15	1.5	10.34	0.12	6.607	0.0102	0.0234
Cw1	2	13.3	121	11.8	1.45	0.017	0	1	0.5467
Cw3	5	33.3							
Cw6	1	6.7	10	1	7.24	0.057	0.75	0.3864	0.1486
Cw7	5	33.3	157	15.3	2.76	0.212	2.394	0.1218	0.0.98

Table 3. Serological HLA types in wheat allergy

Antigen HLA	Workers (n=15)		Normal Japanese (n=898)		Relative risk	δ	Chi square	p Value	Fisher one tall
	number	gene frequency (%)	number	gene frequency (%)					
DR1	2	13.3	49	5.5	2.67	0.083	0.563	0.4529	0.2024
DR2	7	46.7							
DR4	4	26.7	205	22.8	1.23	0.05	0.002	0.9672	0.4614
DRw6	6	40							
DR 9	3	20	117	13	1.67	0.08	0.166	0.6839	0.3142
DRw10	1	6.7	5	0.6	12.76	0.061	1.673	0.1959	0.0949
DRw12(5)	2	13.3							
DRw52	7	46.7							
DRw53	6	40							
DQw1	8	53.3	410	45.6	1.36	0.141	0.109	0.741	0.608
DQw3	4	26.7	169	18.8	1.57	0.1	0.191	0.6622	0.312
DQw4	3	20	134	14.9	1.43	0.06	0.033	0.8559	0.3963
DQw6(w1)	7	46.7							
DQw7(w3)	3	20	137	15.2	1.39	0.056	0.021	0.8852	0.4107

percentages and genotypes as gene frequencies (gf), calculated as follows[7].

$gf = 1 - \sqrt{1 - Pf}$ $Pf = C/N$

(C : gene positive case N : Total number Pf : Phenotype frequency)

Results

I ; Serological type

The frequencies of the Aw33 (w19), B44 (12) and Bw67 serotypes were significantly higher in wheat flour RAST-positive cases compared to controls. A2 and Bw52 also increased, but the differences did not reach statistical significance. No signifidcant differences in HLA-DR and HLA-DQ were observed between the two groups (Table 2, 3). In haplotype frequency, 4 of 10 workers were found to have A33-B44 haplotype, and 3 of 10 workers had A2-B52. The DR4-DQ4 and DR9-DQ3 haplotypes were each found in 3 of 10 workers.

Ten of the 15 workers with positive RAST scores for wheat underwent HLA typing.

II ; Genotype

1. Genotyping of HLA-DPB1 alleles

Eight subjects were positive for DPB1*0510 (gf=0.553), 4 subjects for DPB1*0201 (gf=0.225), 3 subjects for DPB1*0401 (gf=0.163) and 3

Table 4. HLA class II alleles in wheat allergy (DPB1)

Allele	Workera (n=10)		Normal Japanese (n=4183)		Relative risk	δ	Chi square	p Value	Fisher one tall
	number	gene frequency (%)	number	gene frepuency (%)					
DPB1*0501	8	55.3	262	39	2.38	0.464	0.624	0.4295	0.2192
DPB1*0201	4	22.5	156	20.8	1.12	0.042	0	1	0.5513
DPB1*0401	3	16.3	39	4.8	4.17	0.228	2.668	0.1024	0.0648
DPB1*0901	3	16.3	69	8.6	2.17	0.162	0.489	0.4842	0.2275
DPB1*0202	1	5.1	37	4.5	1.14	0.013	0	1	0.6094

Table 5. HLA class II alleles in wheat allergy (DQB1)

Alleie	Worker (n=10)		Normal Japanese (n=307)		Relative risk	δ	Chi square	p Value	Fisher one-tail
	number	gene frequency (%)	number	gene frequency (%)					
DQB1*0604	5	29.3	34	5.7	8.03	0.438	10.232	0.0014	0.0035
DQB1*0601	4	22.5	112	20.4	1.16	0.056	0	1	0.5304
DQB1*0602	3	16.3	32	5.3	3.68	0.219	2.048	0.1524	0.0861
DQB1*0301	2	10.6	87	15.3	0.63	−0.116	0.048	0.8259	0.4333
DQB1*0302	1	5.1	47	8	0.62	−0.063	0	0.9898	0.537
DQB1*0303	1	5.1	0	0	<<	<<	7.206	0.0073	0.0315
DQB1*0401	1	5.1	76	13.2	0.34	−0.196	0.485	0.4863	0.2559
DQB1*0402	1	5.1	15	3.4	2.16	0.054	0	1	0.4088

Odds ratio of infinity (division by zero) are indicated by <<

Table 6. HLA class II alleles in wheat allergy (DRB1)

Alleie	Worker (n=10)		Normal Japanese (n=493)		Rilative risk	δ	Chi square	p Value	Fisher one-tail
	number	gene frequency (%)	number	gene frequency (%)					
DRB1*1302	5	29.3	70	7.4	6.04	0.417	7.281	0.007	0.0089
DRB1*1502	4	22.5	67	9.2	3.11	0.271	1.968	0.1606	0.0676
DRB1*1501	3	16.3	65	6.8	2.82	0.194	1.15	0.141	0.141
DRB1*0405	2	10.6	116	12.5	1.74	0.085	0	1	0.5719
DRB1*1406	1	5.1	17	1.7	0	−0.016	0.06	0.8069	0.3077
DRB1*0410	1	5.1	10	1	5	0.008	0.377	0.539	0.2
DRB1*1201	1	5.1	38	3.9	1.33	0.025	0	1	0.5572
DRB1*0901	1	5.1	126	13.7	0.32	−0.209	0.568	0.4511	0.2356

subjects for DPB1 ＊0901 (gf=0.163). DPB1＊0202 was recognized in only one subject (gf=0.051) (Table 4). No significant differences were observed among the frequency these alleles, although the frequency of DPB1＊0401 was higher than the control frequency.

2. Genotyping of HLA-DQB1 alleles

Five subjects were positive for DQB1＊0604 (gf=0.293), 4 subjects for DQB1＊0601 (gf=0.225) and 3 subjects for DQB1＊0602 (gf=0.163). DQB1＊0301 was recognized in two subjects (gf=0.106), and DQB1＊0302, DQB1＊0303, DQB1＊0401 and DQB1＊0402 in only one subject each (gf=0.051) (Table 5). DQB1 ＊0604 and DQB1＊0303 were higher than controls.

3. Genotyping of HLA-DRB1 alleles

Five subjects were positive for DRB1＊1302 (gf=0.293), 4 subjects for DRB1＊1502 (gf=0.225), 3 subjects for DRB1＊1501 (gf=0.163) and 2 subjects for DRB1＊0405 (gf=0.106). DRB1＊0406, DRB1 ＊0410, DRB1＊1201 and DRB1＊0901 were observed in only one subject each (gf=0.051) (Table 6). The frequency of DRB1＊1302 was significantly higher than the control frequency.

Four of 10 workers had the DRB1＊1302-DQB1＊0604-DPB1＊0201 haplotype, and 3 of 10 workers had DRB1＊1502-DQB1＊0601-DPB1＊0501.

Discussion

The immunodominant sites in allergens as well as the HLA gene products, which present foreign antigens to T cells, require thorough investigation in order to understand the allergen-specific mechanisms underlying the development of certain allergies. The ultimate goal of such research is to identify workers at risk for developing allergic symptoms if exposed to allergens in their workplace, based on analysis of HLA gene products.

Several reports concerning the relation between HLA and atopic disease have been published. Sasazuki et al reported HLA-DQw3 to be positively correlated with Japanese cedar allergy[8]. T-cell responses to the short ragweed allergen Amb a V were reported to be related to DRα/β1 1502[9]. The grass pollen allergen Lol p III is related to the HLA-D gene[10][11]. Previous analysis has established that HLA-DRB1, HLA-DRB3, and HLA-DRB5 encoded gene products may present house dust mite antigens[12]~[15].

Our serological HLA typing data revealed an increase in the frequency of serotype Aw33 (w19) among the stujects. A33 and B44 were reportedly increased in a patient with atopic dermatitis[16]. Four of 10 workers had haplotype A33-B44, although the frequency of this haplotype is reportedly high in Japan. In wheat allergy cases, other antigens, such as mites, house dust, Japanese cedar, and α-amylase and papain used as baking additives, may also contribute to the symptoms. Thus, Aw33 is recognized not only in wheat flour allergy cases but also in atopic disease, suggesting that it is related to allergies other than wheat flour allergy.

The human HLA DRβ, DQα, DQβ and DPβ chains show marked polymorphism, which may contribute to the variety of allergic manifestations occuring in different individuals.

HLA-D region loci are reportedly the restriction elements which present foregin antigen. In the mite, it is reported that DRw52 supertypic specificities may be the restriction elements presenting antigen[14], but we observed no apparent relation between wheat allergy DR in the present study.

The frequency of DPB1＊0401 tended to be higher in the study group compared with controls, but the difference was not significant. In mite allergy, an apparent relation of Der f II to DPB1＊0401 was reported but was not statistically significant. A significant relation was also reported for DPB1＊0401 in patients with hyper IgE, and it was suggested to be related to various antigens such as cats, dogs and alternaria, suggesting that there is a motif common to several allergens[17]. These data suggest the DPB1

may be involved in the atopic mechanism, although not with wheat flour allergy.

DQB1＊0604 was significantly higher in sugjects with wheat allergy in this study, suggesting that it may play a role in wheat flour allergy. DQB1＊0602 and DQB1＊0303 also appeared to be increased. The DQB1＊0302 frequency, which was low in patients with atopic dermatitis[16], in our cases was lower than that in controls, but the difference did not reach statistical significance.

DRB1＊1502 was higher in wheat allergy subjects than in controls, but the difference was not a significant degree. DRB1＊1502 was also reported to contribute, to some extent, to the T-cell response to Der f II, although this allele is common in Japanese subjects[18].

It is very difficult to determine the site of the alleles responsible for wheat allergy since many wheat RAST-positive workers are also positive for other allergens. Some have allergic symptoms but none of the HLA types reported to be related to either the antigen or the disease. In this study, we indentified some alleles which may be involved in wheat flour allergy. Since individuals having one of these HLA types did not always khae an allergic reaction to wheat flour, it seems likely that several epitopes may be involved in the development of wheat flour allergy. It is also possible that the genes which determines disease specificity is near an HLA locus. Co-inheritance of such a gene, with the HLA type, could explain the apparent relation to the allergic disease.

Acknowledgments

We thank Dr. Tohru Hayashi, Tokyo Medical College for valuable suggestions.

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小麦粉喘息とHLAとの相関について

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アレルギー反応は，環境だけでなく遺伝的素因により決定され，アレルゲンはMHCとの複合体の形でリンパT細胞に提示される．HLA（human lymphocyte antigen）の遺伝的多形性は，アレルギー反応の発現に関与していると考えられており，我々はアレルギー反応におけるHLAの関与を検討するため小麦粉アレルギーを生じた症例について検討を行った．対象は製パン，製菓業従事者で小麦粉特異的IgE抗体陽性者15名についてSerological HLA typing（Teraaski-NIH-Standard法）を行った．さらにPCR-RELP法を用いてHLA class allelesのGenotypingを10例で行い，正常日本人との頻度を比較検討した．HLA-Aw33（w19），B-44（12），Bw67の頻度が，小麦粉RAST陽性者で正常コントロール群と比較して有意に多く認められた．HLA calss II AllelesのGenotypingでは，DQB1＊0604，DQB1＊1302が有意に増加していた．有意差は認められなかったが，DPB1＊0401，DQB1＊0602，DRB1＊1502，DRB1＊1502も高頻度傾向であった．ハプロタイプ頻度では，10人中4人がDRB1＊1302-DQB1＊0604-DPB1＊0201であり，10人中3人でDRB1＊1502-DQB1＊0601-DPB1＊0501であった．これらの結果はMHCクラスII分子の小麦粉アレルギーへの果たす役割の重要性が示唆するものと考えられた．

キーワード：小麦粉アレルギー，HLA，職業アレルギー．