

HLA-DRB1*07 may have a susceptibility and DRB1*04 a protective effect upon the development of a sensitization to house dust mite *Dermatophagoides pteronyssinus*

Y.-K. KIM*†, H.-B. OH‡, S.-Y. OH†, S.-H. CHO*†, Y.-Y. KIM*† and K.-U. MIN*†

*Department of Internal Medicine, Seoul National University College of Medicine, †Institute of Allergy and Clinical Immunology, Seoul National University Medical Research Center and ‡Department of Clinical Pathology, Ulsan University College of Medicine, Seoul, Korea

Summary

Background IgE responses to house dust mite-derived allergens seem to be the most important in the development of atopic asthma and rhinitis, but it has been difficult to demonstrate genetic control of the IgE response to the allergens.

Objective This study was undertaken to investigate the association between sensitization to house dust mite, *D. pteronyssinus* (DP), and genotypes of HLA-DRB1 alleles.

Methods DNAs were extracted from two groups of unrelated Koreans: (1) 178 with sensitization to DP; and (2) 99 age-matched non-atopic controls. Genotypes of the HLA-DRB1 alleles were determined using polymerase chain reaction (PCR)-based methods.

Results The frequency of HLA-DRB1*07 was significantly increased in the DP-sensitive subjects compared with the controls (15.7% vs 4.0%, $P = 0.009$). Conversely, the frequencies of HLA-DRB1*04 and *14 were decreased in the DP-sensitive subjects compared with the controls (27.5% vs 45.5%, $P = 0.002$; 13.5% vs 24.2%, $P = 0.02$). Of the DRB1*04 alleles, DRB1*0403 was significantly decreased in the DP-sensitive subjects compared with the controls (3.9% vs 13.1%, $P = 0.005$). No significant differences were found in the distributions of the other HLA-DRB1 alleles between the two groups.

Conclusion HLA-DRB1*07 may have a susceptibility, and DRB1*04 and DRB1*14 may have a protective effect, upon the development of a sensitization to DP.

Keywords: atopy, sensitization, house dust mite, *Dermatophagoides pteronyssinus*, HLA-DRB1

Clinical and Experimental Allergy, Vol. 31, pp. 110–115. Submitted 12 January 2000; revised 22 February 2000; accepted 29 April 2000.

Introduction

Sensitization to common aeroallergens plays an important role in the pathogenesis of asthma and atopic disorders. Atopic disease requires the synthesis of allergen-specific IgE antibodies, but it has been difficult to demonstrate genetic control of the IgE response. Sensitization to house dust mite has been shown to be of particular importance in the development of asthma in children and bronchial hyperresponsiveness in young adults [1,2].

The HLA gene complex is known to contribute to the genetic susceptibility of individuals to a variety of allergic and autoimmune diseases [3]. The association between HLA-class II molecules and the IgE response was first noted in patients sensitized to small, well-defined antigens, such as the ragweed allergens Amb a 5 and the rye grass allergen Lol p 3 [4–7]. Subsequently, two different studies involving epidemiological investigations, or the use of antigen-specific T-cell lines and clones, have revealed that a number of HLA-DR and DQ genes are able to restrict the recognition of house dust mite allergens [8,9], the olive pollen allergen Ole e 1 [10], the birch pollen allergen Bet v 1 [11], and the major cat allergen Fel d 1 [12]. In

Correspondence: K.-U. Min, Department of Internal Medicine, Seoul National University College of Medicine, 28 Yongon-dong, Chongno-gu, Seoul.

population-based studies, however, investigators have failed to demonstrate a significant association between HLA-class II immune response genes and IgE responsiveness to house dust mite allergens [13–16].

In this study, investigations were undertaken to determine whether sensitization to house dust mite *Dermatophagoides pteronyssinus* (DP) was associated with genotypes of HLA-DRB1 alleles. To test this hypothesis, genotypes of HLA-DRB1 alleles were determined using polymerase chain reaction (PCR)-based methods in subjects sensitized to DP and age-matched non-atopic controls.

Materials and methods

Subjects

One hundred and seventy-eight subjects sensitive to DP and 99 age-matched non-atopic healthy controls were recruited for this study. They were unrelated subjects living in rural areas of Cheju Island, Korea. The mean age of the DP-sensitive subjects was 18.9 (range 13–30) years, with 73 males and 105 females. All of them showed positive skin response to DP, defined as a mean weal size formed by the allergen of 4 mm or larger, and, according to a questionnaire, had rhinitis and/or asthma symptoms. The mean age of the controls was 17.8 (range 13–28) years, with 49 males and 50 females. They had been exposed to the same environment as the DP-sensitive subjects since childhood. They revealed no asthmatic or rhinitis symptoms, and showed a negative skin response to any aeroallergen (mean weal size by any allergen <2 mm). All the subjects gave informed consent and the study protocol was approved by the Ethics Committee of the Seoul National University Hospital.

Skin prick tests

All the subjects underwent skin prick tests using a panel of 11 common aeroallergens (Allergopharma Co., Germany) common in Korea. None of the subjects had received antihistamines orally for 5 days preceding the study. The allergens included *Dermatophagoides pteronyssinus*, *D. farinae*, cat fur, moulds (*Alternaria* spp. and *Aspergillus* spp.), pollens (two mixtures of early blooming and late blooming trees, grass pollen mixture, mugwort and ragweed) and cockroach. A positive control of histamine (1 mg/mL, Allergopharma Co., Germany), along with a negative control of diluent (Allergopharma Co., Germany), was included in all the tests. Skin prick tests were performed on the volar side of both forearms. Fifteen minutes after the skin prick, the mean weal size formed by the allergens was measured.

Low-resolution analysis of HLA-DRB1 alleles: PCR with sequence-specific oligonucleotide probe (SSOP)

Genomic DNA from peripheral blood mononuclear cells of DP-sensitive subjects and the controls was extracted using the Wizard Genomic DNA Purification Kit (Promega Co., WI, USA) as directed by the supplier.

Low-resolution analysis of HLA-DRB1 alleles was performed using the Dynal RELI™ SSO HLA-DRB test (Dynal A.S., Oslo, Norway). PCR amplification of the second exon of the DRB1 gene was performed in a PCR reaction mixture consisting of: 25 µL DNA (0.5 µg), 50 µL master mix (Tris solution containing 10% glycerol, KCl, dATP, dCTP, dGTP, dUTP, biotinylated primers, AmpErase, AmpliTaq and 0.05% sodium azide) and 25 µg MgCl₂. Amplification in a thermal cycler (Perkin Elmer, CA, USA) was performed, as follows: 35 cycles of 15 s at 95 °C, 45 s at 60 °C and 15 s at 72 °C, after an initial holding process of 2 min at 50 °C. The reactions were terminated at 72 °C after a final elongation process of 5 min at 72 °C.

Pre-warmed (50 °C) hybridization buffer (3 ml) was added to each well, and 70 µL of denatured amplified sample or control added and hybridized at 30 g for 30 min using specific oligonucleotide probes as directed by the supplier. HLA-DRB1 antigens were interpreted using the Dynal FELI™ SSO Pattern Matching Computer Program.

Genotypes of HLADRB1*15, DRB1*16: PCR with sequence-specific primers (SSP)

For samples typed as HLA-DRB1*02 by PCR-SSOP, PCR was performed with two sets of SSPs which were designed to be specific for DRB1*15 and DRB1*16. One sense primer (TCCTGTGGCAGCCTAAGAGG) was DR2-specific, one antisense primer was specific for DR15 (GCTCCGTCACCGCCCGGA) and another antisense primer was specific for DR16 (GCTCCGTCACCGCCCGGT). PCR amplification was performed in a PCR reaction mixture consisting of 25 µL DNA (0.5 µg), 5 µL PCR buffer (50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris-HCl pH 8.3, 0.1 µg/mL gelatin), 100 µmol of each deoxynucleotide, 10 pmol of each of the primers and 0.25 U DNA polymerase (Boehringer Mannheim, CA, USA). Amplification was performed in a thermal cycler (Perkin Elmer, CA, USA) as follows: 35 cycles of 40 s at 95 °C, 40 s at 59 °C and 40 s at 72 °C, after pre-heating for 5 min at 95 °C. Amplified products were resolved on 2.5% agarose gels with ethidium bromide. HLA-DRB1*15 or DRB1*16 were considered to be positive if a 138 bp band was observed.

High-resolution analysis of HLA-DRB1*04: PCR with single strand conformation polymorphism (SSCP)

Samples typed as HLA-DRB1*04 using the PCR-SSOP,

DRB1*04 subtypes (*0401–*0411) were discriminated using PCR-SSCP. A sense primer (GTTCTTGGAGCAGGT-TAAA, base position 17–37) and an antisense primer (CCGCTGCACTGTGAAGCTCT, base position 260–279) were used for PCR amplification of the second exon of the DRB1 gene. PCR was performed in 10 µL of a PCR reaction mixture consisting of 1 mM MgCl₂, 50 mM Tris-HCl (pH 8.3), 10 mM ammonium sulphate, 20 mM of each of dATP, dCTP, dGTP and dTTP, 0.5 mM of each primer (Table 2), 30 ng of template DNA and 0.2 U Taq DNA polymerase (Boehringer Mannheim, CA, USA). They were mixed gently, overlaid with 10 µL of mineral oil, and then subjected to thermal cycling (Perkin Elmer, CA, USA). After pre-heating for 5 min at 95 °C, 30 amplification cycles were performed, followed by incubation for 5 min at 72 °C. Each amplification cycle consisted of two steps: 1 min at 95 °C for denaturing, and 1 min at 58 °C to anneal and polymerize. Amplification of DNAs was verified using 5% polyacrylamide gel electrophoresis and ethidium bromide staining.

SSCP analysis was performed according to the method described by Bannai *et al.* [17] with a few modifications. Briefly, 1 ml amplified solution was mixed with 7 µL denaturing solution (95% formaldehyde, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol FF). The mixtures were denatured for 5 min at 95 °C and immediately cooled on ice; 2 µL of the samples were then applied to 10% polyacrylamide gel (acrylamide:bisacrylamide = 49:1 with 5% glycerol). A minigel electrophoresis apparatus with a constant temperature control system (90 × 80 × 1 mm, AE-6410, ATTO, Japan) was used.

Electrophoresis was carried out in 45 mM Tris-borate (pH 8.0)/1 mM EDTA at 22 °C. Single-stranded DNA fragments in the gel were detected by silver staining (Daiichi Pure Chemicals, Tokyo, Japan). HLA-DRB1 subtypes (DRB1*0401–0411) were identified by comparison with reference DNA DR types (UCLA DNA Reference Panel, UCLA, LA, USA).

Statistical analysis

HLA-DRB1 alleles were determined by direct counting. Allelic frequency (%) was calculated as follows:

$$\text{Allelic frequency} = n/N \times 100$$

where n = number of individuals positive for each genotype and N = total number of individuals tested

The allele frequencies were compared using the χ^2 test, and odd ratio (OR) with a 95% confidence interval (CI) was calculated. When either value was below 5, Fisher's exact test was used. P -values were corrected for the number of comparisons (P_c), which were considered significant when $P_c < 0.05$.

Results

Table 1 shows HLA-DRB1 genotypes in DP-sensitive subjects and non-atopic healthy controls. The frequency of HLA-DR*07 determined by PCR-SSOP showed a significant increase amongst those sensitive to DP, although the statistical significance was less marked [15.7% vs 4.0%; OR = 4.43 (95% CI 1.14–17.16), $P = 0.009$,

Table 1. Frequency of HLA-DRB1 alleles in *Dermatophagoides pteronyssinus*-sensitive subjects and non-atopic healthy controls

DRB1 alleles	DP-sensitive subjects ($n = 178$) Number (%)	Controls ($n = 99$) Number (%)	OR (95% CI)	P	P_c^*
1	32 (18.0)	21 (21.2)		NS	NS
15	34 (19.1)	21 (21.2)		NS	NS
16	1 (0.6)	0		NS	NS
3	10 (5.6)	4 (4.0)		NS	NS
4	49 (27.5)	45 (45.5)	0.46 (0.34–0.63)	0.002	0.03
11	9 (5.1)	3 (3.0)		NS	NS
12	24 (13.5)	14 (14.1)		NS	NS
13	42 (24.6)	18 (18.2)		NS	NS
14	24 (13.5)	24 (24.2)	0.67 (0.37–1.20)	0.02	NS
7	28 (15.7)	4 (4.0)	4.43 (1.14–17.16)	0.009	NS
8	48 (27.0)	20 (20.2)		NS	NS
9	36 (20.2)	18 (18.2)		NS	NS
10	3 (1.7)	1 (1.0)		NS	NS

DP: *D. pteronyssinus*, OR: odd ratio, NS: not significant. * P corrected for the number of alleles (13) tested.

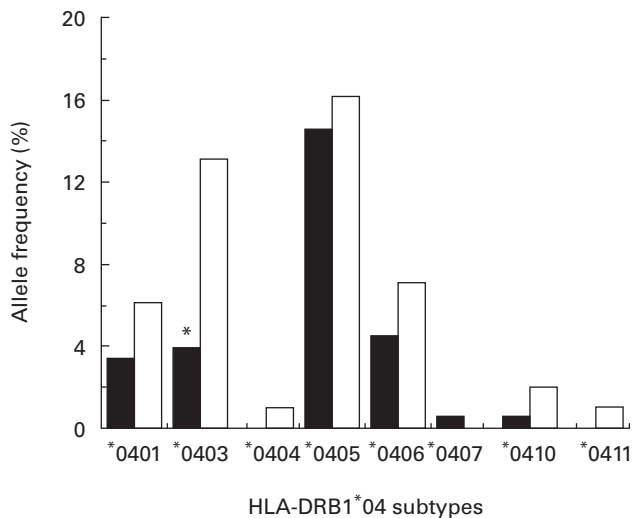


Fig. 1. Allele frequency of HLA-DRB1*04 subtypes by PCR with single strand conformation polymorphism (SSCP). *Significant difference compared with non-atopic controls ($P < 0.05$). (■), *Dermatophagoides pteronyssinus*-sensitive subjects; (□), control.

$P_c = 0.12$]. Conversely, the frequency of HLA-DR*04 determined by PCR-SSOP showed a significant decrease among the DP-sensitive subjects [27.5% vs 45.5%; OR = 0.46 (95% CI 0.34–0.63), $P = 0.002$, $P_c = 0.03$]. The frequency of HLA-DR*14 as determined by PCR-SSOP showed an increase among the DP-sensitive subjects, although the statistical analysis showed no significance [13.5% vs 24.2%; OR = 0.67 (95% CI 0.37–1.20), $P = 0.02$, $P_c = 0.26$]. No significant differences could be ascribed to the other HLA-DRB1 genotype levels in the two groups.

Figure 1 shows distributions of HLA-DRB1*04 subtypes by PCR-SSCP. The lower overall significance regarding DRB1*04 comes from the fact that there is a tendency of all alleles except DRB1*0403 to be significantly under-represented among DP-sensitive subjects (3.9% vs 13.1% $P = 0.005$).

Discussion

The HLA class II (HLA-DR, DQ and DP) molecules are heterodimers (α - and β -chains) with a high proportion of polymorphisms [18]. With the exception of the HLA-DRA molecule, the genes encoding the functional class II molecules are highly polymorphic, with virtually all of the variability localized on the second exon. This exon encodes the amino-terminal extracellular domain, which functions as the antigen-binding site for processed peptides. The DRB1 gene is the most polymorphic of the human class II genes, in which over 120 distinct alleles have been

identified, making it a powerful marker for individual identification [19]. In this study, the HLA-DRB1 genes were targeted.

In an effort to further elucidate the role of immune response genes in the development of atopic disease, many population studies have examined the association between HLA-class II alleles and atopic responses to a number of different allergens. These studies have often yielded conflicting results due to differences in ethnic background, phenotyping definitions and HLA typing methods. Koreans are relatively homogeneous in ethnic background. In particular, the DP-sensitive and control subjects recruited in this study were from the general populations living in rural areas on Cheju island, where they have lived in much the same environments since childhood. In this study, sensitization to DP was defined as positive when the mean weal size formed by the allergen was larger than 4 mm, in order to avoid misdiagnosing any subjects as falsely unaffected. Earlier studies have used serological methods to define HLA types [20], while more recent studies have defined genotypes using PCR-based methods such as PCR-SSOP, PCR-SSP and PCR-SSCP [21]. The PCR-based method has many advantages over the more traditional techniques used for HLA typing, i.e. serology and the mixed lymphocyte reaction [21]. In this study, genotypes of HLA-DRB1 alleles were determined using PCR-SSOP low-resolution analysis, followed by DRB1*15 and DRB1*16 using PCR-SSP, and finally DRB1*04 subtypes using PCR-SSCP high-resolution analysis.

Following the original observations of Levine *et al.* [22], a number of genetic association studies have been conducted to investigate the relationships between particular HLA class II types and specific allergen responsiveness in case-control studies [4–7]. When low-molecular-weight, highly purified and characterized subfractions of allergen were investigated, associations were shown between HLA class II types and elevated IgE levels to short ragweed (*Ambrosia artemisiifolia*) pollen allergens. Further investigations have suggested that DR $\alpha\beta$ 1 heterodimers were the principal HLA class II molecules mediating the presentation of Amb a 5 [23]. Other purified allergens, which have been found to be associated with HLA class II allergens, include rye grass (Lol p 1) and olive (Ole e 1) [7,10].

A family study demonstrated a significant linkage between sensitization to house dust mite and haplotypes of HLA class II genes [13]. However, the case/control population studies did not reveal any association between sensitization to house dust mite and any particular allele of HLA class II genes [13–16]. The negative effects of HLA class II gene polymorphism on the risk of sensitization to whole complex allergens, such as crude DP allergens, may be due to the obscuring effects of the vagaries of allergen exposure, the complexity of crude allergens [24], or the

relatively low number of individuals included [13–16]. In this study, a relatively large number of individuals, who have been living in much the same environment since childhood, were recruited. The results of the study revealed that DP sensitization was negatively associated with HLA-DRB1*04 (particularly with DRB1*0403) and positively with DRB1*07. However, the possibility of an association between HLA-DQ alleles and sensitization to house dust mite cannot be precluded, because HLA-DRB1*04 and DRB1*07 have a linkage disequilibrium with HLA-DQB*04 and DQB*02, respectively, in Korean families [25].

The balance between TH₁ and TH₂ cell activity seems crucial for the control of the immune response. Chronic rheumatoid arthritis (RA) has been suggested to be TH₁-cell predominated [26], whereas atopic diseases was found to be TH₂-cell directed [27]. Hong *et al.* [28] revealed that the frequency of HLA-DR4 was significantly increased in Korean RA patients compared with controls (61% vs 29%; RR = 3.7, *P* < 0.0001). Interestingly, the results of the present study show that levels of HLA-DRB1*04 alleles are lower among DP-sensitive subjects compared with non-atopic healthy controls. This reversed pattern relationship between atopy and RA suggests that the HLA-DRB1 genes may be linked to protection from, or susceptibility to the two diseases, and that the atopic population may be quite separate from the population at risk of developing RA.

In conclusion, this study shows that distributions of HLA-DRB1 alleles in atopic subjects with sensitization to house dust mite have a profile which is the reverse of that found in RA patients. Furthermore, HLA-DRB1*07 may indicate a susceptibility to, and DRB1*04 and *14 may afford protection from the development of sensitization to DP.

Acknowledgements

This study was supported by a research grant (98-HMP-1–0001) from the Ministry of Health and Welfare, Republic of Korea.

References

- 1 Bousquet J, Chané P, Lacoste JY *et al.* Eosinophilic inflammation in asthma. *N Engl J Med* 1990; 323:1033–9.
- 2 Cartier A, Thompson NC, Frith PA, Roberts R, Hargreave FE. Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway calibre. *J Allergy Clin Immunol* 1982; 70:170–7.
- 3 Kostyu DD, Amos DB. The HLA complex: Genetic polymorphism and disease susceptibility. In: Scriver CR, Beaudet AL, Sly WS, Valled D eds. *The Metabolic Basis of Inherited Disease*. New York: McGraw-Hill. 1989: 225.
- 4 Huang SK, Zwollo P, Marsh DG. Class II major histocompatibility complex restriction by human T cell responses to short ragweed allergen, Amb a V. *Eur J Immunol* 1991; 21:1469–73.
- 5 Goldstein R, Yang WH, Drouin MA, Karsh J. Studies of the HLA class II alleles involved in human responses to ragweed allergen *Ambrosia artemisiifolia* V (Ra5S) and *Ambrosia trifida* V (Ra5G9). *Tissue Antigens* 1992; 39:122–7.
- 6 Marsh DG, Freidhoff LR, Ehrlich-Kautzky E, Bias WB, Roebber M. Immune responsiveness to *Ambrosia artemisiifolia* (short ragweed) pollen allergen Amb a VI (Ra6) is associated with HLA-DR5 in allergic humans. *Immunogenetics* 1987; 26:230–6.
- 7 Ansari AA, Shinomiya N, Zwollo P, Marsh DG. HLA-D gene studies in relation to immune responsiveness to a grass allergen Lol p III. *Immunogenetics* 1991; 33:24–32.
- 8 O'Hehir RE, Lamb JR. Direct evidence for a functional role of HLA-DRB1 and -DRB3 gene products in the recognition of *Dermatophagoides* spp. (house dust mite) by helper T lymphocytes. *Int Immunol* 1990; 2:885–92.
- 9 Marsh DG, Blumenthal MN, Ishikawa T, Ruffili A, Sparholt S, Friedhoff LR. HLA and Specific Immune Responsiveness to Allergens In: Tsuji K, Aizawa M, Sasazuki T eds. Oxford: Oxford University Press, 1991: 765.
- 10 Cardaba B, Vilches C, Martin E *et al.* DR7 and DQ2 are positively associated with immunoglobulin E response to the main antigen of olive pollen (Ole e I) in allergic patients. *Human Immunol* 1993; 38:293–9.
- 11 Sparholt SH, Georgsen J, Madsen HO, Svendsen UG, Schou C. Association between HLA-DRB3*0101 and immunoglobulin E responsiveness to Bet v I. *Hum Immunol* 1994; 39:76–8.
- 12 Young RP, Dekker JW, Wordsworth BP *et al.* HLA-DR and HLA-DP genotypes and immunoglobulin E response to common major allergens. *Clin Exp Allergy* 1994; 24:431–9.
- 13 Torres-Galvan MJ, Quirarte J, Blanco C *et al.* Linkage of house dust mite allergy with the HLA region. *Ann Allergy Asthma Immunol* 1999; 82:198–203.
- 14 O'Brien RM, Tait BD, Varney MD, Thomas WR. HLA-DPB1 alleles in house dust mite allergic patients. *Eur J Immunogenet* 1999; 26:281–4.
- 15 Stephan V, Kuehr J, Seibt A *et al.* Genetic linkage of HLA-class II locus to mite-specific IgE immune responsiveness. *Clin Exp Allergy* 1999; 29:1049–54.
- 16 Stephan V, Schmid V, Frischer T *et al.* Mite allergy, clinical atopy and restriction by HLA class II immune response genes. *Pediatr Allergy Immunol* 1996; 7:28–34.
- 17 Bannai M, Tokunaga K, Imanishi T *et al.* Discrimination of human HLA-DRB1 alleles by PCR-SSCP (single-strand conformation polymorphism) method. *Eur J Immunogenetics* 1994; 21:1–9.
- 18 Unanue ER. Cellular studies on antigen presentation by class II MHC molecules. *Curr Opin Immunol* 1992; 4:63–9.
- 19 Rammensee HG, Friede T, Stevanovic S. MHC ligands and peptide motifs. *First Listing Immunogenetics* 1995; 41:178–228.
- 20 Blumenthal M, Marcus-Bagley D, Awdeh Z, Johnson B, Yunis e alper CA. HLA-DR, 2 [HLA-B7, SC31, DR21] and

- [HLA-B8, SC01, DR3] haplotypes distinguish subjects with asthma from those with rhinitis only in ragweed pollen allergy. *J Immunol* 1992; 148:411–6.
- 21 Kimura A, Sasazuki T. Eleventh International Histocompatibility Workshop reference protocol for the HLA-DNA typing technique. In: Tsuji K, Aizawa M, Sasazuki T eds. *HLA 1991 Proceedings of the Eleventh International Histocompatibility Workshop and Conference, Vol 1*. Oxford: Oxford University Press. 1992: 397.
- 22 Levine BB, Stember RH, Fontino M. Ragweed hayfever: genetic control and linkage to HLA haplotypes. *Science* 1972; 178:1201–3.
- 23 Zwollo P, Kautzky EE, Scharf SJ, Ansari AA, Erlich HA, Marsh DG. Sequencing of HLA-D in responders and non-responders to short ragweed allergen, Amb a V. *Immunogenetics* 1991; 33:141–51.
- 24 Joost-van-Neerven R, van t'Hof W, Ringrose JM, Aalberse RC, Wierenga EA, Kapsenberg ML. T cell epitopes of house dust mite major allergen Der p II. *J Immunol* 1993; 151:2326–35.
- 25 Park MH, Hwang YS, Park KS *et al.* HLA haplotypes in Koreans based on 107 families. *Tissue Antigens* 1998; 51:347–55.
- 26 Panayi GS, Lanchbury JS, Kingsley GH. The importance of the T cell in initiating and maintaining the chronic synovitis of rheumatoid arthritis. *Arthritis Rheum* 1992; 35:729–35.
- 27 Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 1996; 17:138–46.
- 28 Hong GH, Park MH, Takeuchi F *et al.* Association of specific amino acid sequence of HLA-DR with rheumatoid arthritis in Koreans and its diagnostic value. *J Rheumatol* 1996; 23:1699–703.