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ORIGINAL ARTICLE

The role of human leukocyte antigen tissue groups in hepatitis B virus vaccination in Turkey

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Background/Purpose: Between 5% and 10% of the vaccinated population responds less well to standard vaccination schedules irrespective of hepatitis B virus (HBV) vaccination. This manuscript aims at describing possible correlation of different major histocompatibility complex (MHC) Class-I and MHC Class-II haplotype to anti-HBV humoral responsiveness following HBV vaccination.
Materials and Methods: The study was conducted on 944 vaccinated hospital staff members and concentrated on the 38 nonresponders as defined by enzyme-linked immunosorbent assay (ELISA) results. In order to define significance of the different haplotypes from the nonresponders, their frequency was compared to the frequency of the same haplotype in 18 randomly selected responders. Human leukocyte antigen (HLA)-A and HLA-B antigens were typed among total mononuclear cells using a standard two-stage microlymphocytotoxicity test. The typing method of HLA Class-II is based on a technique that involves amplification of the second exon of different HLA Class-II genes by PCR.
Results: Positive correlations were found between four HLA-DR (HLA-DRB1*04X, DRB1*0401X, DRB1*11/13, and DRB1*0401X0201) haplotypes and nonresponders but there was a negative correlation with one Class-I (HLA-B13).
Conclusion: This study suggested that certain HLA types are associated with nonresponsiveness to vaccination. The different HLA of ethnic groups should also be kept in mind when evaluating

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the response to hepatitis vaccination. The different HLA gene frequencies of ethnic groups should be examined in further large-scale population-based studies.

Introduction

Hepatitis B vaccination remains the best tool available to prevent and control hepatitis B virus (HBV) infection and related diseases, which still threaten about a million people worldwide. Recombinant HBV vaccines achieve seroprotection in more than 90% of the vaccinated healthy adult population. The hepatitis B vaccine is considered to be highly immunogenic and has a good safety record. About 5—10% of the vaccinated population responds less well to standard vaccination schedules irrespective of hepatitis B vaccination.[1,2]

Genetic factors are implicated in the response of normal individuals to hepatitis B vaccine. Various constitutional and behavioral factors have been described that could have a negative influence on the immune response and play a role in nonresponsiveness; age, male sex, obesity, anti-human immunodeficiency virus (HIV) seropositivity, and smoking habits.

The major histocompatibility complex (MHC), termed the human leukocyte antigen (HLA) region and encoded on chromosome 6 in humans, is involved in the regulation of immune responses. General genetic factors and, more specifically, polymorphisms of the MHC have also been linked to variation in immune responses. Some studies have linked the presence or absence of several HLAs to poor responsiveness after vaccination.[3]

MHC genes may also control the capability of the humoral response to vaccination. The DPB1*0501 allele is associated with improved antibody responses to vaccination with a malaria sporozoite antigen in Thai individuals.[4] Hemodialyzed patients either do not respond to or develop very low antibody titers against significant numbers of hepatitis B vaccines.[5,6] Ethnic differences in HLA phenotypes may also show hypo- or nonresponsiveness to HBV vaccination. Weak responsiveness to hepatitis B vaccine is associated with the DR3 and/or DR7 alleles,[7—11] when they are present on the extended haplotypes HLA B8-DR3-SC01 and HLA B44-DR7-FC31, in Caucasians. Another report has shown that nonresponsiveness to the hepatitis B vaccine is strongly associated with the extended HLA haplotype B44; DRB1*0701, DQB1*0202 genotype.[12] In Japanese individuals, the Bw54-DR4-DQW4 haplotype has been connected with poor vaccine responses.[13—15] Nevertheless, most studies regarded a limited number of alleles and nonresponders, meaning that the results were rarely statistically significant.

In this study we sought to investigate efficacy of MHC and some genetic factors on nonresponsiveness of hepatits B vaccination in healthcare workers of our hospital (Gulhane Military Medical Academy Hospital, Ankara, Turkey).

Methods

Participants

The study was conducted with 38 participants (26 were male) who were nonresponders to hepatitis B vaccination among the 944 vaccinated members of the hospital staff. The hospital's total number of employees is around 6000. The nonresponse rate was 4%. Study procedures were approved by the institutional review board prior to study initiation. Participants were selected in the routine vaccination program of health officers. Routine informed consent for clinical procedures was obtained by the infectious disease specialist. All participants were assessed for smoking habits, obesity, and noncommunicable familial chronic diseases such as renal, cardiac, hematologic, or hepatic diseases. Samples were obtained over a 10-month period. The serological profiles for HBV surface antigen (HBsAg), anti-HBsAg antibody (anti-HBs), hepatitis B e-antigen, anti-hepatitis B e-antigen, and total anti-HBV core antibodies of the participants were found to be negative. Included in the exclusion criteria was the simultaneous administration of any other vaccine, or participation in another clinical trial at the time of administration of hepatitis B vaccine.

None of the 38 participants, who were enrolled from medical outpatient services and ranged in age from 20 years to 47 years, were clinically obese, indicated chronic kidney disease, or had histories of smoking. All individuals consented to be included in the study. A control group was randomly selected in which 18 individuals were responders to heptitis B vaccination, with antibody titers $>$100 IU/mL.

Standard hepatitis B vaccination program

Three doses of S-subunit recombinant hepatitis B vaccine (20 μg of Engerix B; GlaxoSmithKline, Rixensart, Belgium) are routinely given to all new staff members working at the hospital after the determination of absence of HBsAg and anti-HBs. The protocol involves the application of vaccine intramuscularly in the deltoid muscle at 0 months, 1 month, and 6 months. Measurement of serum levels of antibody to HBsAg serum samples were obtained from vaccinated individuals 4 weeks after the third vaccination. The levels of antibody responses specific for HBsAg were assayed by radioimmunoassay using a commercial test system (Axsym system, Abbott Laboratories, Abbott Park, IL, USA). The individuals who had $<$10 IU/mL anti HBs were accepted as nonresponders, and those who had $>$100 IU/mL anti-HBsAg after the third dose of vaccine, were enrolled as responders.

Determination of HLA Class-I

HLA-A and HLA-B antigens were typed on total mononuclear cells by the standard two-stage microlymphocytotoxicity test using extended incubation times of Dyna Beads as previously described.[16]

Determination of HLA Class-II

DNA was extracted from peripheral granulocytes using standard methods. The HLA Class-II (DRB1, DRP1, and DQB1) was determined with INNO-LIPA DRB (Line Probe Assay, Catalog numbers 80336, 80340, and 80344; Innogenetics, Ghent, Belgium) as developed by Buyse et al.[17] This typing method is based on a technique that involves an amplification of the second exon of different HLA Class-II genes by PCR. The amplified DNA was hybridized to membrane-bound sequence-specific oligonucleotides, and subsequently visualized by using an alkaline phosphate-based reaction.

Statistical analysis

In order to determine heterogeneity values for all alleles detected at each locus, Fisher's exact tests were used, and the level of significance was set at $p < 0.05$. The double-sided statistical method was used, initially for absence of any foresight. Odds ratios were calculated with Woolf's formula and reported with 95% confidence intervals, and these ratio values reflect the likelihood of carrying a particular HLA allele in the nonresponder group compared with the responder group.

Results

Fifty-nine types of antigens were found to possess HLA-Class-I antigens as detected by the Dyna Beads HLA-Class-I method. Seventy types of antigens were positive for HLA-Class-II antigens variable by line probe assay reverse hybridization. The distribution of HLA Class-I and HLA Class-II antigens in both case and control groups are presented in Tables 1 and 2. The average age of the responders was 37 ± 6.9 years. The male-to-female ratio in the responder ($n = 18$) population was 4:1, while in the nonresponder population it was 2:1. The frequency of specific HLA types among responders and nonresponders are summarized in Tables 1 and 2.

Linked to HLA alleles and anti-HBs antibody production

The alleles at each HLA locus that either positively or negatively contributes to the anti-HBs antibody production

Table 1 Human leukocyte antigen (HLA) Class I antigens in nonresponders and responders to hepatitis B virus vaccination

HLA Class-I haplotypes	Nonresponders $n = 38$ (%)	Responders $n = 18$ (%)	p
HLA BW6	30 (78.94)	15 (83.33)	0.5007
HLA BW4	23 (60.52)	10 (55.55)	0.7410
HLA CW4	12 (31.57)	4 (22.22)	0.3478
HLA B35	11 (28.94)	8 (21.05)	0.3815
HLA B13	15 (39.47)	1 (5.55)	0.0072*
HLA A2	15 (39.47)	7 (38.88)	0.6016
HLA CW6	9 (23.68)	3 (16.66)	0.4114
HLA A11, HLA A26(10)	7 (18.42)	2 (5.26)	0.3926
HLA A24(9)	7 (18.42)	5 (27.77)	0.8730
HLA A11,	7 (18.42)	4 (22.22)	0.7600
HLA A3, HLA A24(9)	7 (18.42)	3 (16.66)	0.5950
HLA B51(5),HLA CW7	6 (18.75)	2 (11.11)	0.4917
HLA A26(10)	5 (13.15)	4 (22.22)	0.8926
HLA B60(40),HLA CW5	4 (10.52)	1 (5.55)	0.4792
HLA A33(19),HLA A1,HLA CW3,HLA CW5	4 (10.52)	2 (11.11)	0.7111
HLA A1	4 (10.52)	4 (22.22)	0.1590
HLA A29(19)	3 (7.89)	2 (11.11)	0.8171
HLA B57(17),	3 (7.89)	0 (0.00)	0.3043
HLA A29(19), HLA B7, HLA B41 HLA B73, HLA CW1	3 (7.89)	1 (5.55)	0.6144
HLA A25(10)	2 (5.26)	2 (11.11)	0.2928
HLA A23(9)	2 (5.26)	0 (0.00)	0.4565
HLA A68(28),HLA A32(19), HLA A30(19), HLA B50(21), HLA B42, HLA B58(17), HLA B63(15)	2 (5.26)	1 (5.55)	0.7608
HLA A28(68), HLA A(31)19, HLA B52(5), HLA B18	1 (2.63)	2 (11.11)	0.2392
HLA A32, HLA B38(16), HLA B44(12), HLA B8, HLA B55(22), HLA B24(9), HLA B72(70), HLA B56(22), HLA B49(21), HLA B73(15), HLA B14,HLA B51(5)	1 (2.63)	1 (5.55)	0.4441
HLA B48, HLA B58(16), HLA B51(22), HLA B63(5)	0 (0.00)	1 (5.55)	0.3214

* $p < 0.05$.

Table 2 Human histocompatibility leukocyte antigen (HLA) Class II antigens in nonresponders and responders to hepatitis B virus vaccination

HLA Class-II haplotypes	Nonresponders, $n = 38$ (%)	Responders, $n = 18$ (%)	p
DPB1*0401X0201	8 (21.05)	9 (50.00)	0.0309*
DRB1*07X	8 (21.05)	2 (11.11)	0.3058
DQB1*020102X	7 (18.42)	0 (0.00)	0.0544
DRB1*11X	6 (18.75)	2 (11.11)	0.4917
DRB1*13	5 (13.15)	1 (5.55)	0.3633
DQB1*0301, DQB1*0301X	5 (13.15)	0 (0.00)	0.1314
DRB1*04	4 (10.52)	2 (11.11)	0.6367
DRB1*01X	4 (10.52)	1 (5,55)	0.4792
DRB1*01X, DQB1*0302, DQB1*0501X	4 (10.52)	0 (0.00)	0.2010
DRB1*04X	3 (7.89)	8 (44.44)	0.0027*
DPB1*0401X2301	3 (7.89)	1 (5.55)	0.6144
DPB1*2301X5101, DQB1*0201X	3 (7.89)	0 (0.00)	0.3043
DRB1*0401X	2 (5.26)	5 (27.77)	0.0292*
DRB1*16, DPB1*0401X0402	2 (5.26)	2 (11.11)	0.3856
DRB1*14 DPB1*0301X DPB1*1011	2 (5.26)	1 (5.55)	0.6957
DRB1*03 DRB1*03X DRB1*12 DPB1*0301 DPB1*0402X5101 DPB1*2301 DPB1*0501-3801 DQB1*06011X DQB1*0402X DQB1*0305X	2 (5.26)	0 (0.00)	0.4565
DRB1*11/13	1 (2.63)	4 (22.22)	0.0327*
DPB1*1701	1 (2.63)	2 (11.11)	0.2392
DRB1*16X DRB1*11 DRB1*16/15 DRB1*07 DRB1*08 DPB1*5101	1 (2.63)	1 (5.55)	0.5435
DRB1*2X, DPB1*12/4101, DPB1*0402X, DPB1*5001X0401 DPB1*0402, DPB1*2012X0301, DPB1*0402X2901, DPB1*2501X4601, DPB1*3301X0402, DPB1*0402X, DPB1*0401X3801, DPB1* 1601X, DPB1*1601, DQB1*0401, DQB1*6011,12, DQB1*0603X, DPB1*0401X0402	1 (2.63)	0 (0.00)	0.6786
DRB1*15;	0 (0.00)	2 (11.11)	0.0994
DRB1*13/14; DRB1*14X; DRB1*15X; DPB1*1401(2); DPB1*1501; DPB1*0401; DPB1*0402X4101	0 (0)	1 (5.55)	0.3214
DRB1*13/3; DPB1*0201X2; DPB1*11011; DPB1*1301X; DPB1*0401X0501; DPB1*20123201; DPB1*1011,12X; DPB*2301X5101; DPB*0501,3801X; DP*0402X02011; DP*0402X0201112320	0 (0)	0 (0.00)	1

* $p < 0.05$.

with statistical significance and the partial correlation coefficients of these alleles are listed in Tables 1 and 2. In the HLA-A and HLA-B loci, HLA-B13 contributed negatively to the anti-HBs antibody response ($p = 0.072$). HLA-B13 was more frequent in the nonresponders (15/38, 39.5%) compared to the responders (1/18, 5.6%). In the HLA-DRB1 locus, HLA-DRB1*04X, DRB1*0401X, DRB1*11/13, and DRB1*0401X0201 positively contributed to the response. HLA-DRB1*04X (3/38, 7.89%), DRB1*0401X (2/38, 5.26%), DRB1*11/13 (1/38, 2.63%), and DRB1*0401X0201 (8/38, 21.05%) were less frequent in the nonresponders, whereas the distribution of the same alleles were more frequent in the responders, 44%, 27,7%, 22.22%, and 50%, respectively.

Haplotypes with the highest frequencies among nonresponders (compared to responders) were HLA B60 (40), HLA CW5, DRB1*01X, DRB1*07X, and DRB1*13 (2 times more frequent). By contrast, HLA A28 (68), HLA A (31)19, HLA B52 (5), HLA B18, and DPB1*1701 were 2.5 times more frequent among the responders. However, these differences in frequencies were not significant.

Discussion

Although the effects of HLA types of immune responses could not be fully defined, certain HLA types have been found to be associated with nonresponsiveness to vaccination.[18–20] Some risk factors that have been associated with nonresponsiveness to HBV vaccine include male sex, obesity, increasing age, cigarette smoking, the gluteal administration of the vaccine,[21,22] chronic renal failure, and diabetes.[23] The relationship of HBV vaccination response with HLA types is under investigation and there are a limited number of studies on the subject in Turkey.

The generation of a successful anti-HBs response requires cooperation between HBsAg-specific T and B

lymphocytes. It can be hypothesized that a reduction or absence of one or both of these components may lead to a defective or absent anti HBs response.[24] The lymphocytes from most of the good responders to HBV vaccine proliferated *in vitro* upon stimulation with HBsAg particles, whereas the lymphocytes from the majority of intermediate or poor/nonresponders generally do not react upon stimulation with HBsAg. It is possible that nonresponders are defective in the presentation of HBsAg.

On the basis of HLA-A, HLA-B, and HLA-C phenotype frequencies, the Turkish population can be compared to European whites, Japanese, blacks, and also Greek and Tunisian populations.[25,26] Turks appear to have patterns similar to European Caucasoid and Greek populations for HLA-A phenotypes.[27] In the Japanese, however, HLA-A1 is lower than in other populations. The most common A and B locus antigens for Turkish and white Europeans are A2, A9, B5, B35, and B12, respectively.[28] In contrast to those findings most commonly observed locus antigens for our study population were HLA BW6, HLA BW4, and DPB1. Although the study population included in the previous study[28] mainly consisted of people living in the west part of Turkey, the majority of this study participants came from central and eastern parts of Turkey. From that point of view we speculate that geographical difference may be associated with the different HLA haplotypes found in the current study. In the Japanese population, B5, B40, and B15 are the most common B locus antigens. In blacks, B12, B5, and B7 are the more common B locus antigens. The less common B locus antigen is B15 for Turkish, B14 for white Europeans, B13 for Greeks and blacks, and B18 for the Japanese.[29]

The weak responsiveness to HBsAg vaccine is associated with the DR3 and/or DR7 alleles,[7–11] when they are present on the extended haplotypes HLA B8-DR3-SC01 and HLA B44-DR7-FC31 in Caucasians. Our results reveal that extended haplotypes HLA-DRB1*04X, DRB1*0401X, DRB1*11/13, and DRB1*0401X0201 are significantly associated with antibody response to the HBV vaccine. The haplotypes described above have not been reported in other investigations. HLA A1 was at a high frequency in responders such as DQ2, DQ3, DR3, B44, DR4, and DR53 in other investigations. In this study it was found that HLA A1 frequencies were not significantly different in both responders and nonresponders, consistent with other reports.[30,31]

The HLA B13 haplotype was associated with nonresponsiveness to vaccination in our participants, which to-date has not been reported by others. Certain HLA haplotypes such as B46 and B15 were higher in nonresponders as previously reported.[31] However, the frequency of HLA B15 is considerably low in the Turkish general population, and the coding for this antigen was not detected in our participants.[29] Our results indicate that the different HLA gene frequencies of ethnic groups should be considered for further larger population based responders and nonresponders.

Limitations and restrictions

The control group included a limited number of participants, did not cover the generally Turkish population (only the Central Anatolia of Turkey), and was of small sample size.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Ethics approval

Study procedures were approved by the institutional review board.

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References

1. Coates T, Wilson R, Patrick G, André F, Watson V. Hepatitis B vaccines: assessment of the seroprotective efficacy of two recombinant DNA vaccines. *Clin Ther* 2001;**23**: 392—403.
2. Assad S, Francis A. Over a decade of experience with a yeast recombinant hepatitis B vaccine. *Vaccine* 1999;**18**:57—67.
3. Desombere I, Van der Wielen M, Van Damme P, Stoffel M, De Clercq N, Goilav C, et al. Immune response of HLA DQ2 positive subjects, vaccinated with HBsAg/AS04, a hepatitis B vaccine with a novel adjuvant. *Vaccine* 2002;**20**:2597—602.
4. Stephens HA, Brown AE, Chandanayingyong D, Webster HK, Sirikong M, Longta P, et al. The presence of the HLA Class-II allele DPB1*0501 in ethnic Thais correlates with an enhanced vaccine-induced antibody response to a malaria sporozoite antigen. *Eur J Immunol* 1995;**25**:3142—7.
5. Crosnier J, Jungers P, Couroucé AM, Laplanche A, Benhamou E, Degos F, et al. Randomised placebo-controlled trial of hepatitis B surface antigen vaccine in French haemodialysis units: II, Haemodialysis patients. *Lancet* 1981;**1**:797—800.
6. Stevens CE, Alter HJ, Taylor PE, Zang EA, Harley EJ, Szmuness W. Hepatitis B vaccine in patients receiving hemodialysis. Immunogenicity and efficacy. *N Engl J Med* 1984;**311**: 496—501.
7. Craven DE, Awdeh ZL, Kunches LM, Yunis EJ, Dienstag JL, Werner BG, et al. Nonresponsiveness to hepatitis B vaccine in health care workers. Results of revaccination and genetic typings. *Ann Intern Med* 1986;**105**:356—60.
8. Weissman JY, Tsuchiyose MM, Tong MJ, Co R, Chin K, Ettenger RB. Lack of response to recombinant hepatitis B vaccine in nonresponders to the plasma vaccine. *JAMA* 1988; **260**:1734—8.
9. Pol S, Legendre C, Mattlinger B, Berthelot P, Kreis H. Genetic basis of nonresponse to hepatitis B vaccine in hemodialyzed patients. *J Hepatol* 1990;**11**:385—7.
10. Varla-Leftherioti M, Papanicolaou M, Spyropoulou M, Vallindra H, Tsiroyianni P, Tassopoulos N, et al. HLA-associated non-responsiveness to hepatitis B vaccine. *Tissue Antigens* 1990;**35**:60—3.
11. Alper CA, Kruskall MS, Marcus-Bagley D, Craven DE, Katz AJ, Brink SJ, et al. Genetic prediction of nonresponse to hepatitis B vaccine. *N Engl J Med* 1989;**321**:708—12.
12. McDermott AB, Zuckerman JN, Sabin CA, Marsh SG, Madrigal JA. Contribution of human leukocyte antigens to the

antibody response to hepatitis B vaccination. *Tissue Antigens* 1997;**50**:8—14.
13. Watanabe H, Matsushita S, Kamikawaji N, Hirayama K, Okumura M, Sasazuki T. Immune suppression gene on HLA-Bw54-DR4-DRw53 haplotype controls nonresponsiveness in humans to hepatitis B surface antigen via CD8+ suppressor T cells. *Hum Immunol* 1988;**22**:9—17.
14. Watanabe H, Okumura M, Hirayama K, Sasazuki T. HLA-Bw54-DR4-DRw53-DQw4 haplotype controls nonresponsiveness to hepatitis-B surface antigen via CD8-positive suppressor T cells. *Tissue Antigens* 1990;**36**:69—74.
15. Hatae K, Kimura A, Okubo R, Watanabe H, Erlich HA, Ueda K, et al. Genetic control of nonresponsiveness to hepatitis B virus vaccine by an extended HLA haplotype. *Eur J Immunol* 1992; **22**:1899—905.
16. Terasaki PI, Bernoco D, Park MS, Ozturk G, Iwaki Y. Microdroplet testing for HLA-A, -B, -C, and -D antigens. The Phillip Levine Award Lecture. *Am J Clin Pathol* 1978;**69**:103—20.
17. Buyse I, Decorte R, Baens M, Cuppens H, Semana G, Emonds MP, et al. Rapid DNA typing of Class-II HLA antigens using the polymerase chain reaction and reverse dot blot hybridization. *Tissue Antigens* 1993;**41**:1—14.
18. Desombere I, Willems A, Leroux-Roels G. Response to hepatitis B vaccine: multiple HLA genes are involved. *Tissue Antigens* 1998;**51**:593—604.
19. Langö-Warensjö A, Cardell K, Lindblom B. Haplotypes comprising subtypes of the DQB1*06 allele direct the antibody response after immunisation with hepatitis B surface antigen. *Tissue Antigens* 1998;**52**:374—80.
20. Durupinar B, Okten G. HLA tissue types in nonresponders to hepatitis B vaccine. *Indian J Pediatr* 1996;**63**:369—73.
21. Arslanoğlu I, Cetin B, Işgüven P, Karavus M. Anti-HBs response to standard hepatitis B vaccination in children and adolescents with diabetes mellitus. *J Pediatr Endocrinol Metab* 2002;**15**: 389—95.
22. Leroy V, Bourliere M, Durand M, Abergel A, Tran A, Baud M, et al. The antibody response to hepatitis B virus vaccination is negatively influenced by the hepatitis C virus viral load in patients with chronic hepatitis C: a case-control study. *Eur J Gastroenterol Hepatol* 2002;**14**:485—9.
23. Tele SA, Martins RM, Lopes CL, dos Santos Carneiro MA, Souza KP, Yoshida CF. Immunogenicity of a recombinant hepatitis B vaccine (Euvax-B) in haemodialysis patients and staff. *Eur J Epidemiol* 2001;**17**:145—9.
24. Zuckerman JN. Hepatitis B third-generation vaccines: improved response and conventional vaccine non-response—-third generation pre-S/S vaccines overcome non-response. *J Viral Hepat* 1998;**5**(Suppl. 2):13—5.
25. Ayed K, Bardi R, Gebuhrer L, Gorgi Y, Betuel H. HLA-A, B, C and DR antigens in a sample of the Tunisian population. *Tissue Antigens* 1987;**29**:225—31.
26. Alper CA, Awdeh Z, Raum D, Yunis EJ. Complement genes of the major histocompatibility complex (complotypes), extended haplotypes and disease markers. *Biochem Soc Symp* 1986;**51**:19—28.
27. Pachoula-Papasteriadis C, Ollier W, Cutbush S, Economidou J, Festenstein H. HLA antigen and haplotype frequencies in Greeks. *Tissue Antigens* 1989;**33**:488—90.
28. Uyar FA, Dorak MT, Saruhan-Direskeneli G. Human leukocyte antigen-A, -B and -C alleles and human leukocyte antigen haplotypes in Turkey: relationship to other populations. *Tissue Antigens* 2004;**64**:180—7.
29. Aydogan K, Karadogan SK, Akdag I, Tunali S. HLA class I and Class-II antigens in Turkish patients with chronic ordinary urticaria. *Clin Exp Dermatol* 2006;**31**:424—9.
30. Stachowski J, Kramer J, Füst G, Maciejewski J, Baldamus CA, Petrányi GG. Relationship between the reactivity to hepatitis B virus vaccination and the frequency of MHC class I, II and III alleles in haemodialysis patients. *Scand J Immunol* 1995;**42**: 60—5.
31. Yap I, Chan SH. A new pre-S containing recombinant hepatitis B vaccine and its effect on non-responders: a preliminary observation. *Ann Acad Med Singapore* 1996;**25**:120—2.