

HLA Class I and Class II Genotyping in Patients with Chronic Urticaria

Müge Çoban^a Teoman Erdem^b Şevki Özdemir^b İbrahim Pirim^c
Mustafa Atasoy^b Mevlit İkbal^d

^aDermatology Section, Rize State Hospital, Rize, and ^bDermatology Department, ^cMedical Biology Department and ^dGenetic Department, Atatürk University, Medical School, Erzurum, Turkey

Key Words

Chronic urticaria · Autoimmunity · HLA

Abstract

Objectives: Chronic urticaria (CU) is a common disease in which pathogenesis is unclear and which is resistant to therapy. Recent investigations have indicated that autoimmunity plays a role in nearly one third of CU patients. The present study aimed to investigate the relationship between human leukocyte antigen (HLA) class I and class II antigens and immune pathogenesis of CU. **Methods:** HLA class I and class II antigens were investigated in 40 patients diagnosed with CU, utilizing serologic techniques and polymerase chain reactions. The study was performed between October 2005 and May 2006. Further HLA typing in patient subsets was done depending on the response of patients to intradermal injection of autologous serum. About 30 healthy and genetically unrelated individuals formed the control group for evaluation. **Results:** The results revealed that HLA-B44 frequency was significantly higher (25%) in the patient group as compared with the matched control group (3.33%) ($p = 0.033$, OR = 9.667). There was no significant difference in HLA-A allelic distribution between the patient and control groups. In the genotyping of class II HLA alleles, HLA-DRB1*01 (25%) ($p = 0.033$, OR = 9.667) and HLA-DRB*15 (25%) ($p = 0.033$, OR = 9.667) were predominant alleles in the patient group. **Conclusion:** The association of HLA-B44, HLA-

DRB1*01 and HLA-DRB*15 alleles with idiopathic CU suggests that there is a genetic component in the pathogenesis of CU.

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Introduction

Chronic urticaria (CU) manifests through transient and itchy papules and plaques with individual lesions lasting less than 24 h, and affecting patients for 6 weeks or longer [1, 2]. Markers of autoimmunity may be found in a significant proportion of patients with CU [3]. A commonly encountered marker of autoimmunity, histamine-releasing IgG autoantibodies against the high-affinity receptor for IgE (Fc ϵ RI), or against IgE itself, occurs in 25–45% of patients with CU [2, 4–6]. When the serum from a CU patient was reinjected intradermally, it produced a wheal at the site of injection in approximately 60% of the cases [2, 4].

Human leukocyte antigen (HLA) molecules are polymorphic membrane glycoproteins, which can be expressed on the surface of all nucleated cells (HLA class I molecules) and cells especially involved in the immune response (HLA class II molecules). HLA molecules have been shown to be involved in the homing of lymphocytes, providing mechanisms for skin trying to make lymphocytes migrate selectively into the dermis and epidermis.

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1018–2438/08/1472–0135\$24.50/0

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Correspondence to: Prof. Dr. Teoman Erdem
Dermatology Department
Atatürk University, Medical School
P.K.:2, TR–25171 Erzurum (Turkey)
Tel. +90 442 231 2373, Fax +90 442 236 1301, E-Mail mterdem@hotmail.com

Table 1. Phenotype frequencies of HLA class II alleles

HLA antigen	Patients (n = 40)	Controls (n = 30)	χ^2_Y	p	OR	CI (95%)
A*1	9 (25%)	3 (10%)	1.108	0.292	2.613	0.641–10.647
A*2	11 (27.5%)	5 (16.6%)	0.609	0.435	1.897	0.580–6.201
A*3	9 (25%)	5 (16.6%)	0.091	0.763	1.452	0.431–4.885
A*11	6 (15%)	4 (13.3%)	0.000	1.000	1.147	0.293–4.488
A*23	4 (10%)	1 (3.33%)	0.363	0.547	3.222	0.341–30.426
A*24	11 (27.5%)	6 (20%)	0.196	0.658	1.517	0.489–4.707
A*25	1 (2.5%)	3 (10%)	0.668	0.414	0.231	0.023–2.338
A*26	3 (7.5%)	2 (6.6%)	0.000	1.000	1.135	0.178–7.258
A*29	1 (2.5%)	2 (6.6%)	0.065	0.798	0.359	0.031–4.156
A*30	1 (2.5%)	2 (6.6%)	0.065	0.798	0.359	0.031–4.156
A*31	1 (2.5%)	1 (3.33%)	0.000	1.000	0.744	0.045–12.390
A*32	7 (17.5%)	2 (6.6%)	0.959	0.327	2.970	0.570–15.466
A*33	0	5 (16.6%)	4.887	0.027 ^a	–	–
A*66	1 (2.5%)	0	0.000	1.000	–	–
A*68	5 (12.5%)	4 (13.3%)	0.000	1.000	0.929	0.227–3.801
A*69	1 (2.5%)	1 (3.33%)	0.000	1.000	0.744	0.045–12.390

^a p < 0.05.

More autoimmune diseases have been shown to be associated with alleles of the HLA region than with any other genetic region. Several different class II genes have been directly implicated in the pathogenesis of human and experimental autoimmunity [2, 7].

The present study aimed to investigate the relationship between HLA class I and class II antigens and immune pathogenesis of CU.

Material and Methods

The study included 40 Turkish CU patients diagnosed both clinically and histopathologically at the University Hospital, Erzurum (Turkey) and 30 healthy Turkish controls. The study was performed between October 2005 and May 2006. All patients afflicted with CU disease were seen as outpatients for a period of nearly 1 year. The healthy control subjects were unrelated to each other or to the patients. Blood examinations were done in all subjects.

The autologous serum skin test was performed as described earlier. In short, 50 g/ml of undiluted autologous serum, sterile saline (negative control) and histamine (10 µg/ml in saline, positive control) were injected with disposable tuberculin syringes fitted with 26-gauge needles into the dermis of the volar aspect of the right forearm at a distance of at least 5 cm. A reading of the wheal was taken after 30 min. A wheal and flare of over 2 mm greater in diameter than those of the controls were considered positive [2, 4, 8].

EDTA blood was used to prepare DNA from peripheral blood cells applying the Sigma GenElute Kit according to the manufacturer's instructions.

Low-resolution typing for HLA-A, B, C and HLA-DR/DQ was executed by means of the PCR-SSP method, using SSP HLA class I generic DNA Typing Tray, lot 002 and SSP HLA class II generic DNA Typing Tray, lot 004 (One Lambda, Canoga Park, Calif., USA) according to the manufacturer's instructions. Additional HLA typing was done in patient subsets classified by the response of patients to intradermal injection of autologous serum.

χ^2 analysis with Yates' correction was performed and then both standard p value and the significance of an association were evaluated. The degree of association was calculated by the odds ratio (OR). SPSS 11.5 for Windows was used to make all statistical calculations.

Results

Forty CU patients (26 females, 14 males, 15–60 years of age; mean of 34.09 ± 7.25 years) and 30 healthy Turkish controls (20 females, 10 males, 22–58 years of age; mean of 31.02 ± 3.05 years) were included in the study. The mean $\pm$ SD duration of CU was 2.10 ± 1.56 years, and the range was from 2 months to 15 years. There was angioedema in 20% of the patients. Twenty-five patients underwent a positive autologous serum skin test.

The phenotype frequencies of HLA-A alleles defined by the PCR-SSP method are shown in table 1. Distribu-

Table 2. Phenotype frequencies of HLA-B alleles

HLA antigen	Patients (n = 40)	Controls (n = 30)	χ^2_Y	p	OR	CI (95%)
B*7	3 (7.5%)	1 (3.33%)	0.050	0.824	2.351	0.232–23.802
B*8	1 (2.5%)	2 (6.6%)	0.065	0.798	0.359	0.031–4.156
B*13	2 (5%)	2 (6.6%)	0.000	1.000	0.737	0.098–5.554
B*14	1 (2.5%)	1 (3.33%)	0.000	1.000	0.744	0.045–12.390
B*18	4 (10%)	4 (13.3%)	0.003	0.957	0.722	0.165–3.156
B*27	3 (7.5%)	5 (16.6%)	0.662	0.416	0.405	0.089–1.851
B*35	14 (35%)	8 (26.6%)	0.233	0.629	1.481	0.525–4.180
B*38	2 (5%)	0	0.268	0.605	–	–
B*39	3 (7.5%)	2 (6.6%)	0.000	1.000	1.135	0.178–7.258
B*41	2 (5%)	1 (3.33%)	0.000	1.000	1.526	0.132–17.663
B*44	10 (25%)	1 (3.33%)	4.550	0.033 ^a	9.667	1.163–80.377
B*49	3 (7.5%)	0	0.878	0.349	–	–
B*50	1 (2.5%)	0	0.000	1.000	–	–
B*51	7 (17.5%)	4 (13.3%)	0.020	0.887	1.379	0.364–5.222
B*52	6 (15%)	2 (6.6%)	0.497	0.481	2.471	0.462–13.212
B*55	0	1 (3.33%)	0.021	0.884	–	–
B*57	4 (10%)	0	1.596	0.206	–	–
B*58	1 (2.5%)	0	0.000	1.000	–	–
B*60	1 (2.5%)	0	0.000	1.000	–	–
B*62	1 (2.5%)	0	0.000	1.000	–	–
B*63	1 (2.5%)	1 (3.33%)	0.000	1.000	0.744	0.045–12.390
B*65	0	1 (3.33%)	0.021	0.884	–	–

^a p < 0.05.

tion of HLA-A alleles in patient and control groups did not appear to be significant as assessed by p value (p < 0.05) and OR value (nonsignificant).

HLA-B44 allele frequency was, however, 25% in the patient group, which was statistically significant compared to the control group (3.33%) (p = 0.033, OR = 9.667). The frequencies of HLA-B alleles are given in table 2.

In the genotyping of HLA, class II alleles are given in table 3. HLA-DRB1*01 (25%) (p = 0.033, OR = 9.667) and HLA-DRB*15 (25%) (p = 0.033, OR = 9.667) were predominant alleles in the patient group. Autologous serum tests were positive and negative in patients with CU and did not represent any difference regarding HLA class I and II alleles. The results have not been shown.

Discussion

The immunological characterization of CU is still incomplete. CU is considered to be an autoimmune disease in about one third of patients [9–13]. A histamine-releasing anti-Fc ϵ RI alpha autoantibody has been determined

in about 30% of patients with CU. In addition, in a minority of patients, histamine release is mediated through anti-IgE autoantibodies [2]. An association of thyroid autoimmunity with CU was reported in several studies. The number of CU cases among thyroid patients is significantly higher than among healthy controls and patients without thyroid autoantibodies [14–16].

CU and its genetic relation in terms of HLA have not yet been clarified definitely. The mechanism underlying the association between the inheritance of particular HLA alleles and disease susceptibility has not been well defined so far. This mechanism is responsible for an individual disease association, and it is important to distinguish between autoimmune, immune complex-mediated and nonimmune diseases. These are important distinctions because they will help in the interpretation of HLA associations. With autoimmune disease, the pathogenesis results from the direct effects of a specific immune response (an autoantibody or an autoreactive T cell response) against the host tissue. CU cannot be defined purely in this group. Immune complex diseases involve autoantibodies, and, therefore, CU does not seem to be in

Table 3. Phenotype frequencies of HLA-II alleles

HLA antigen	Patients (n = 40)	Controls (n = 30)	χ^2_Y	p	OR	CI (95%)
DRB1*01	10 (25%)	1 (3.33%)	4.550	0.033 ^a	9.667	1.163–80.377
DRB1*04	13	6 (20%)	0.796	0.372	1.926	0.663–5.860
DRB1*07	2 (5%)	4 (13.3%)	0.642	0.423	0.342	0.058–2.007
DRB1*08	2 (5%)	1 (3.33%)	0.000	1.000	1.526	0.132–17.663
DRB1*10	1 (2.5%)	0	0.000	1.000	–	–
DRB1*11	7 (17.5%)	3 (10%)	0.237	0.626	1.838	0.433–7.813
DRB1*12	2 (5%)	0	0.268	0.605	–	–
DRB1*13	9 (22.5%)	4 (13.3%)	0.443	0.506	1.887	0.521–6.841
DRB1*14	1 (2.5%)	3 (10%)	0.668	0.414	0.231	0.023–2.338
DRB1*15	10 (25%)	1 (3.33%)	4.550	0.033 ^a	9.667	1.163–80.377
DRB1*16	5 (12.5%)	0	2.374	0.123	–	–
DRB1*17	5 (12.5%)	0	2.374	0.123	–	–

^a p < 0.05.

this category. The third type of HLA-associated diseases does not have any known immune component in its pathogenesis, and CU may be evaluated here. Overall, the association of HLA and CU is not the direct cause of the disease, but specific HLA allele frequencies could be negative despite the existence of the disease.

The role of HLA association in the mechanism of autoimmunity is not very clear. But the immunological response may be different due to disparate antigens determined genetically and linked to the MHC complex. In a study performed by Haas et al. [9], the expression of MHC class II molecules is involved in diverse types of urticaria, particularly in persistent whealing reactions.

A study performed by Van Neste and Boillence [17] of HLA class I antigens in 27 patients with CU did not show any significant differences between patient and control groups. Ossi et al. [18] found a meaningfully increased frequency of HLA-A2, HLA-B44, and HLA-B35 antigens in a clinically well-defined group of 13 CU patients as compared with the control population but HLA class II antigens did not show any significant difference between patients and control groups. O'Donnell et al. [2] found a meaningfully increased frequency of DR4 and DQ8 allele in a clinically well-defined group of 100 CU patients. In addition, they found that HLA-DR15 and its associated allele DQ6 were significantly less frequently associated with the disease.

There are two published studies from Turkey about HLA typing of patients with CU. In Aydogan et al. [19] HLA Bw4 and HLA DQ1 antigens were determined as

significantly higher in CU. In another study, Oztas et al. [20] found an increased frequency of HLA DR4 in CU patients in comparison with healthy controls. In the two studies, the autologous serum skin test was not studied. The results suggest regional differences.

In conclusion, association of HLA with CU is important. It seems that some HLA alleles are able to bind self-peptides and elicit T cell responses to self-antigens because of amino acid motifs in the crucial regions of their HLA molecules. Association of HLA-B44, HLA-DRB1*01 and HLA-DRB*15 alleles with CU suggests that there is a genetic component in the pathogenesis of CU.

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