

HLA Status in Patients with Chronic Spontaneous Urticaria

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Key Words

Allele frequencies · Antigens · Chronic urticaria · HLA · IgE

Abstract

Chronic spontaneous urticaria (CSU) is the most common form of chronic urticaria. A considerable amount of data supports an immunological basis for CSU. Some research has focused on the association between chronic urticaria and specific human leukocyte antigen (HLA) alleles. The aim of this study was to investigate the HLA status of Polish patients diagnosed with CSU. **Methods:** The standard complement-dependent microlymphocytotoxicity assay and PCR amplification with sequence-specific primers were used to analyze HLA alleles in 115 patients diagnosed with CSU, and the results were compared to those from 162 healthy, genetically unrelated individuals. **Results:** Among the HLA-A alleles, A-33 occurred significantly more often in the control group ($p < 0.01$). Analysis of the HLA-B allele frequencies revealed the prevalence of the B44 antigen in the study group ($p < 0.0001$). Frequencies of HLA-C alleles and HLA-DQ did not differ significantly between the groups. Among the HLA class II alleles, DRB1*04 was observed significantly more often in the study population ($p < 0.001$), mainly in the autoimmune subtype of urticaria. **Conclusion:** HLA alleles may be involved in CSU development or play a protective role in CSU.

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Introduction

Chronic spontaneous urticaria (CSU) is characterized by the presence of transient itchy wheals and/or angioedema affecting patients for 6 weeks or longer (not including predominantly physical, aquagenic, cholinergic or exercise-induced anaphylaxis/urticaria, or contact urticaria). CSU includes different subtypes of urticaria, all of which can coexist in a single patient. Although it affects 0.1–3.0% of the population, the pathogenic mechanisms of CSU are not well understood. The presence of functional autoantibodies, infectious factors (e.g. *Helicobacter pylori*) or pseudoallergic reactions may induce CSU. Therefore, urticaria comprises a variety of inflammatory and immunological reactions. However, present research emphasizes the role of mast cells as the primary effector cells in chronic urticaria and IgG histamine-releasing autoantibodies against high-affinity IgE receptors in about 30% of the patients. Less frequently, IgE-dependent urticaria has been confirmed [1].

The relationship between HLA polymorphisms and autoimmune dermatological diseases, such as dermatitis herpetiformis, pemphigus vulgaris and alopecia areata, has been confirmed in several studies [2–5]. Some studies support the hypothesis that HLA alleles may be involved in the pathogenesis of chronic urticaria during the initiation of immune responses [6]. A link between

chronic urticaria and HLA type II, HLA-DR and HLA-DQ alleles has been reported. The increase in the frequency of these alleles has been observed in many autoimmune diseases. Some findings have indicated that certain HLA alleles can be responsible for urticaria, while others have a protective role in chronic urticaria [7]. On the other hand, there is still no association between specific HLA alleles and defined subtypes of chronic urticaria [8].

The present study investigates the relationship between HLA class I and II alleles and the immune pathogenesis of chronic spontaneous urticaria.

Patients and Methods

Patients and Controls

The study group consisted of 115 patients (57 females and 58 males) at a mean age of 35.07 ± 6.21 (range 18–69) years with CSU (according to the EAACI/GA²LEN/EDF guidelines) [9]. The diagnosis of CSU was based on a routine clinical examination and the results of the following tests: antinuclear antibodies (Abs), complement compounds, anti-*H. pylori* Abs, history of atopy, total serum IgE, autologous serum skin test (ASST), and thyroid hormones and autoantibodies. Patients with physical, aquagenic, cholinergic and exercise-induced as well as IgE-mediated urticaria were excluded [9]. We isolated two subtypes of CSU in our patients: autoimmune chronic urticaria and non-autoimmune chronic urticaria. Autoimmune chronic urticaria was confirmed by a positive ASST and the presence of any autoantibody. ASST was performed according to Sabroe et al. [10]. Samples of 50 µl autologous serum, 10 µg/ml histamine and 0.9% sterile saline were separately injected into the volar aspect of the forearm. Any red weal reaction was measured at 30 min. The area of the weal was calculated from the measurement of two perpendicular diameters (d_1 and d_2) according to the formula $\pi[(d_1 + d_2)/4]^2$. A positive test was defined as a red serum-induced weal with a diameter ≥ 1.5 mm versus a saline-induced response at 30 min [10]. The presence of autoantibodies such as thyroid anti-peroxidase Ab (TPO), thyroglobulin Ab (TG), cell nuclei Ab (ANA), smooth muscle Ab (ASMA), mitochondrial Ab (AMA) and granulocyte cytoplasm Ab (cANCA and pANCA) were assessed by indirect immunofluorescence (Euroimmune, Lübeck, Germany). Positive results were interpreted according to the manufacturer's instructions.

The control group included 162 healthy subjects (84 females and 78 males) with a mean age of 33.87 ± 5.33 (range: 21–66) years.

HLA Antigen Typing

HLA class I alleles were evaluated in peripheral blood samples using the standard complement-dependent microlymphocytotoxicity assay. The typical 168 commercial antisera (Biotest: Behring, Marburg, Germany) were used to determine specificities. HLA class II genotyping of genomic DNA was performed by PCR amplification using sequence-specific primers (DQ-DR Combi tray, GenoVision, Vienna, Austria).

Data were analyzed for statistical significance using the Fisher exact test with Bonferroni correction and the Spearman correlation test using Statistica 8.0 software. The strength of the association was estimated by calculating the odds ratio (OR) and 95% confidence interval (CI), which was generated by simple binary logistic regression analysis. $p < 0.01$ was considered to be statistically significant.

Results

Our results indicated that 84 patients (73%) had autoimmune urticaria. Angioedema was found in 31% of the patients. Phenotype frequencies of HLA-A, -B, -Cw, -DQA1, -DQB1 and -DRB1 are shown in table 1. The HLA-A33 allele frequency was significantly elevated in the study group (10.8%) compared to the control group (1%; $p < 0.01$, OR = 9.76, 95% CI: 0.56–13.441). Analysis of the frequencies of HLA-B alleles showed that the B44 allele was also more prevalent in the study group ($p < 0.0001$, OR = 7.68, 95% CI: 0.231–8.551). The phenotype frequencies of the HLA-C and HLA-DQ alleles did not differ significantly between the groups.

Among the HLA class II alleles, the DRB1*04 allele frequency was statistically significantly different compared to the control group ($p < 0.001$, OR = 2.98, 95% CI: 0.456–6.781). Further assessment of the associations between HLA alleles revealed a significant negative correlation between alleles HLA-B44+ and A-34– in patients with CSU ($p = 0.002$, Spearman correlation test $R = -0.87$). In patients with the autoimmune subtype of urticaria, the phenotype frequency of the DRB1*04 allele was significantly more common (48%) than in the other study patients with urticaria (1.2%; $p < 0.00001$, OR = 2.62, 95% CI: 0.142–5.891). No other statistically significant differences or correlations were found.

Discussion

Many studies have investigated the etiology of chronic urticaria. Some of these have focused on the autoimmune basis of the disease. The presence of IgG antibodies to the high-affinity IgE receptor on mast cells and basophils was clearly confirmed by an autologous serum skin test. However, in many patients, the mechanisms governing regional mast cell or basophil activation are still unknown. The possible relationship between urticarial reactions and HLA alleles involved in autoimmune responses should be explored [1, 4, 7, 11].

Table 1. The frequency of HLA-A, -B, -Cw, -DQA1, -DQB1 and -DRB antigens in the study groups

	CSU group	Control group	Fisher's exact test, p value		CSU group	Control group	Fisher's exact test, p value
HLA-A (<i>p</i> = 0.000286)	(n = 230)	(n = 324)		HLA-Cw (<i>p</i> = 0.2524)	(n = 177)	(n = 249)	
A*01	29 (12.6)	27 (8.3)	0.115794	Cw*01	14 (7.9)	22 (8.8)	0.860076
A*02	55 (23.9)	82 (25.3)	0.764548	Cw*02	18 (10.2)	15 (6.0)	0.141134
A*03	41 (17.8)	39 (12.0)	0.065667	Cw*03	18 (10.2)	38 (15.2)	0.146237
A*11	12 (5.2)	23 (7.1)	0.478856	Cw*04	41 (23.1)	53 (21.3)	0.722252
A*23	6 (2.6)	7 (2.2)	0.780350	Cw*05	19 (10.7)	41 (16.5)	0.119580
A*24	19 (8.3)	38 (11.7)	0.203618	Cw*06	3 (1.7)	8 (3.2)	0.374372
A*25	1 (0.4)	4 (1.2)	0.408458	Cw*07	39 (18.6)	33 (13.2)	0.018628
A*26	7 (3.0)	6 (1.9)	0.401857	Cw*08	3 (1.7)	4 (1.6)	1.000000
A*29	22 (9.6)	32 (9.9)	1.000000	Cw*12	0 (0)	1 (0.4)	1.000000
A*30	12 (5.2)	15 (4.6)	0.841921	Cw*13	18 (10.2)	28 (11.2)	0.754126
A*31	9 (3.9)	6 (1.9)	0.184441	Cw*14	1 (0.6)	2 (0.8)	1.000000
A*33	0 (1)	35 (10.8)	0.003373	Cw*15	0 (0)	0 (0)	0.572849
A*34	1 (0.4)	0 (0)	0.415163	Cw*16	2 (1.1)	1 (0.4)	0.644546
A*43	1 (0.4)	1 (0.3)	1.000000	Cw*17	1 (0.6)	3 (1.2)	0.860076
A*66	3 (1.3)	2 (0.6)	0.653679	Cw*18	0 (0)	0 (0)	0.141134
A*68	1 (0.4)	1 (0.3)	1.000000				
A*74	11 (4.8)	6 (1.9)	0.077095	HLA-DQA1 (<i>p</i> = 0.0113)	(n = 96)	(n = 151)	
HLA-B (<i>p</i> = 0.000002)	(n = 213)	(n = 210)		DQA1*01	35 (36.5)	54 (35.8)	1.000000
B*07	20 (9.4)	23 (11.0)	0.631823	DQA1*02	6 (6.3)	24 (15.9)	0.027102
B*08	14 (6.6)	16 (7.7)	0.708429	DQA1*03	14 (14.6)	20 (13.2)	0.766201
B*13	9 (4.2)	2 (1.0)	0.062082	DQA1*04	15 (15.6)	11 (7.3)	0.054071
B*14	3 (1.4)	10 (4.8)	0.052285	DQA1*05	12 (12.5)	25 (16.6)	0.466210
B*15	6 (2.8)	4 (1.9)	0.751012	DQA1*06	14 (14.6)	17 (11.3)	0.438511
B*18	4 (1.9)	9 (4.3)	0.170319	HLA-DQB1 (<i>p</i> = 0.5204)	(n = 146)	(n = 168)	
B*27	14 (6.6)	20 (9.5)	0.287508	DQB1*02	52 (35.6)	72 (42.9)	0.204380
B*35	1 (0.5)	0 (0)	1.000000	DQB1*03	16 (11.0)	12 (7.1)	0.321002
B*37	13 (6.1)	19 (9.0)	0.274508	DQB1*04	7 (4.8)	9 (5.4)	1.000000
B*39	2 (0.9)	2 (1.0)	1.000000	DQB1*05	18 (12.3)	15 (8.9)	0.359923
B*41	1 (0.5)	0 (0)	1.000000	DQB1*06	53 (36.3)	60 (35.7)	1.000000
B*42	2 (0.9)	2 (1.0)	1.000000	HLA-DRB (<i>p</i> = 0.000011)	(n = 204)	(n = 239)	
B*44	54 (25.4)	6 (2.9)	0.000005	DRB1*01	18 (8.8)	26 (10.9)	0.525732
B*45	0 (0)	1 (0.5)	0.496454	DRB1*03	9 (4.4)	11 (4.6)	1.000000
B*46	1 (0.5)	1 (0.5)	1.000000	DRB1*04	29 (14.2)	1 (0.4)	0.000959
B*49	1 (0.5)	2 (1.0)	0.621452	DRB1*07	14 (6.9)	12 (5.0)	0.425261
B*50	1 (0.5)	1 (0.5)	1.000000	DRB1*08	3 (1.5)	3 (1.3)	1.000000
B*51	0 (0)	1 (0.5)	0.496454	DRB1*09	1 (0.5)	9 (3.8)	0.023904
B*52	5 (2.3)	9 (4.3)	0.290736	DRB1*10	17 (8.3)	12 (5.0)	0.180235
B*53	3 (1.4)	4 (1.9)	0.722666	DRB1*11	15 (7.4)	35 (14.6)	0.016233
B*54	18 (8.4)	20 (9.5)	0.736133	DRB1*12	1 (0.5)	1 (0.4)	1.000000
B*55	16 (7.5)	17 (8.1)	0.857763	DRB1*13	29 (14.2)	33 (13.8)	1.000000
B*56	1 (0.5)	7 (3.3)	0.036242	DRB1*14	34 (16.7)	43 (18.0)	0.801562
B*57	3 (1.4)	7 (3.3)	0.218272	DRB1*15	27 (13.2)	40 (16.8)	0.352298
B*58	10 (4.7)	04 (1.9)	0.172431	DRB1*16	6 (2.9)	10 (4.2)	0.612093
B*59	7 (3.3)	22 (10.5)	0.003655	DRB1*17	1 (0.5)	3 (1.3)	0.628007
B*67	1 (0.5)	1 (0.5)	1.000000				
B*81	1 (0.5)	0 (0)	1.000000				
B*82	2 (0.9)	0 (0)	0.498840				

n = Sum of chromosomes. Statistically significant differences are indicated in italics (*p* < 0.01).

In this study, the authors demonstrated an association between some HLA class I and II alleles and CSU. In patients with CSU, HLA-A33 occurred less frequently, whereas HLA-B44 was more common. The lower incidence of some HLA alleles in CSU patients may indicate their protective role in a healthy population. This phenomenon has been well documented in other diseases. However, there are not sufficient data concerning urticaria. Melo et al. [12] reported the association between extrinsic allergic alveolitis and a decrease in the frequency of the HLA-DRB1*08 and HLA-DQB*04 alleles. There was no clear evidence for such a phenomenon in urticaria. However, Pacor et al. [11] observed that HLA-Cw4 and HLA-Cw7 occurred less frequently in patients with chronic idiopathic urticaria associated with aspirin and/or hypersensitivity to non-steroidal anti-inflammatory drugs. Aydoğan et al. [13] suggested a possible protective role for HLA-A24 in chronic urticaria in a Turkish population. A strict comparison between studies was difficult because of the ethnic differences. For example, HLA-A24 was more frequent in Oriental, Amerindian and Australasian aboriginal populations than in Caucasoid populations [14]. On the other hand, HLA-A33 occurred with comparable frequency in all the major ethnic groups [i.e. approximately 2.74% (0.00–17.50%) in the Caucasoid population] [14]. It is possible that the protective role of the A33 allele against urticaria observed in our study could be confirmed in other ethnic groups [14]. HLA antigens that may be responsible for susceptibility to chronic urticaria were investigated in several studies [6, 15–17]. However, different HLA phenotypes were observed to be associated with chronic urticaria in different studies. The small study populations, excessively homogeneous groups and problems with well-defined subtypes of urticaria (with many subtypes of urticaria coexisting in the same patient) in addition to ethnic differences may in part explain these differences.

HLA class I alleles have been investigated less often than HLA class II alleles in connection with disease predisposition. It has been proposed that the HLA-Bw4 and HLA-DQ1 alleles were positive genetic markers for chronic ordinary urticaria. Pasero et al. [17] suggested that the HLA-B51 antigen played the same role in patients with the urticaria/arthritis syndrome. The correlation between symptomatic dermatographism and HLA class I antigens (HLA-A2, -B16 and -B5) was also noticed in a Spanish population [18]. Our findings support the positive relationship between HLA-B44 and urticaria, consistent with the results described by Pacor et al. [11]. However, those results were from an analysis of patients with chronic idiopathic urticaria associated with aspirin and/

or hypersensitivity to non-steroidal anti-inflammatory drugs. Therefore, their study may involve different types of urticaria. The HLA-B44 allele is more common in the Caucasoid population, with an average frequency of 11.19% (4.60–21.70%) [14].

Most studies have confirmed the relationship between the HLA class II alleles DR, DP and DQ and various types of urticaria or related diseases, such as hereditary angioedema, mastocytosis, anaphylaxis and dermatographism. Moreover, some data indicate an association between other immunological diseases, allergies or drug intolerance and the HLA class II alleles [4, 11]. HLA class II alleles were also proven to be risk or protective factors for sensitization to specific allergens. In a previous study, an increase in the HLA-DRB4 and -DQ8 frequencies and a decrease in HLA-DR15 and -DQ6 allele frequencies were found to be protective factors in a group of 100 patients with chronic urticaria [5].

Since 2000, a few studies have shown a strong association between chronic urticaria and HLA-DRB1, *12, *0901 and DRB4 [13, 19]. The authors found the same correlation as Oztas et al. [6] between the presence of HLA-DRB4 (endogenous peptide L-plastin 581–595) and CSU. The distribution of this allele in the population was not available. However, a strong positive association of the DRB4 allele with CSU, suggesting genetic pathogenesis, does not preclude the involvement of environmental or other genetic factors. Our findings correspond broadly with studies by O'Donnel et al. [5] and Oztas et al. [6], but further investigation in other major ethnic groups is needed. A significant negative correlation between A33– and B44+ was observed in patients with CSU in the present study. Such interdependence between HLA alleles was also noticed in another study [13]. However, Aydoğan et al. [13] described a significant negative correlation of HLA-Bw4+A24– and HLA-DQ1+A24– and a positive correlation of HLA-Bw4+DQ1– alleles in patients with chronic ordinary urticaria. Our results likely differ because of the different ethnic populations analyzed in these studies. To our knowledge, we have observed the first known association between the autoimmune subtype of urticaria and the HLA allele, in contrast to the results of a study by Coban et al. [8]. Further studies will be required to confirm this observation. Nevertheless, these results have proven that the genetic basis of urticaria is complex and probably based on various interactions between HLA alleles.

In conclusion, we assume that HLA alleles are involved in the development of urticaria and may also play a protective role against this disease.

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