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Research Communication

Relationship Between Human Leucocyte Antigen Class I and Class II and Chronic Idiopathic Urticaria Associated With Aspirin and/or NSAIDs Hypersensitivity

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Background. HLA genes play a role in the predisposition of several diseases. The aim was to analyze the prevalence of HLA class I phenotypes and HLA-DRB1* genotype in patients with CIU associated with ASA and NSAIDs hypersensitivity (AICU). *Methods*. 69 patients with AICU, and 200 healthy subjects. *Results*. Subjects with HLA-B44 and HLA-Cw5 antigens were more represented in patients with AICU than in control group. Subjects with HLA-A11, HLA-B13, HLACw4, and HLA-Cw7 antigen were more represented in control group than in patients with AICU. Multiple logistic regression demonstrated an association of HLA-Cw4 and HLA-Cw7 with a lower risk of AICU, whereas carriers of HLA-B44 phenotype had a higher risk of AICU. No differences were found between patients and controls as regards to HLA-DRB1* genotype. *Conclusions*. We observed an association between some HLA class-I antigens and AICU. To the best of our knowledge this is the first description of such association.

INTRODUCTION

Urticaria is a common skin disorder characterized by erythematous, raised skin lesions, usually intensely itching, lasting less than 24 hours, or occasionally even longer. By definition, urticaria of < 6 weeks duration is arbitrarily considered "acute," whereas urticaria recurring > 6 weeks is referred to as "chronic." Generally, 40 to 60% of the patients with chronic urticaria do not have a well-described cause ("ordinary" chronic idiopathic urticaria) [1, 2].

In approximately 60% of patients with chronic urticaria it has been demonstrated that intradermal injection of autologous serum causes a weal and flare response typical of an urticarial lesion, due to the presence of IgG antibodies directed to the α subunit of the high affinity IgE receptor (Fc$\cong$ εRI) on mast cell and basophils (autoreactive chronic urticaria). In other patients, aspirin and/or nonsteroidal anti-inflammatory drugs (NSAIDs) and/or food additives hypersensitivity can aggravate urticaria. However, in many patients the mechanisms causing their urticaria remain obscure [3–5].

The human leucocyte antigen (HLA) genes play a major role in regulating the immune response. HLA class-I and class-II molecules are membrane-bound glycoproteins that bind intracellularly processed antigenic peptides and present them to T cells via T-cell receptors and are encoded by the most highly polymorphic gene family in the human genome [6]. HLA class-I molecules are present on all nucleated cells and platelets and present peptides of endogenous origin to CD8+ T cells, while HLA class-II molecules are expressed on a more restricted range of cell types—including B cells, activated T cells, and the monocyte/macrophage lineage—and

present short peptides of exogenous origin to CD4+ T cells, mainly with helper phenotype. Therefore, polymorphisms of the HLA molecules can modulate the capacity to bound and present different peptides [7].

HLA loci play a well-known role in predisposition to several and different diseases, including rheumatoid arthritis, ankylosing spondylitis, coeliac disease, insulin-dependent diabetes mellitus, multiple sclerosis, atopic disease, and food allergy [8]. Mainly HLA type-II polymorphisms have been associated with such diseases, but also the HLA type-I locus has been found in relation with some pathologies (ie, HLA-B27 and ankylosing spondylitis) [9].

The aim of our study is to analyze the prevalence of HLA class-I phenotypes (HLA A, B, Cw) and HLA-DRB1* genotype in blood from patients affected by chronic idiopathic urticaria associated with aspirin and/or NSAIDs hypersensitivity (AICU).

MATERIALS AND METHODS

Patients

A total of 69 patients (16 males, 53 females; mean age 42.3 ± 16.2 years), affected by AICU, living in North-East Italy, were selected from the outpatient clinic of the Dipartimento di Medicina Clinica, Università di Verona (Italy). AICU was defined as the presence of urticarial lesions recurring > 6 weeks, exacerbated by the administration of aspirin and/or NSAIDs, without any other known secondary causes. In all patients we performed a specific challenge with *oral acetylsalicylic acid (ASA)* (see below). The presence of urticarial skin lesions, with or without angioedema, was clinically confirmed in all patients. Our institutional policy and the ethical committee in our institution do not require that an ethics committee authorize the study. However, institutional policy requires the patient's written informed consent for us to perform the study; we obtained consent in every case. The study was carried out during the period between January 2002 and December 2003.

Patients with physical urticaria, positive skin test to autologous serum, or food additives hypersensitivity were excluded from the study [10–13]. The control group was represented by 200 healthy subjects, enrolled in the bone marrow donors register (97 males, 103 females; mean age 29.9 ± 4.9 years), from the same geographic region, sex- and age-matched. No subject of the control group was affected by atopic/allergic diseases and/or acute or chronic urticaria.

Oral acetylsalicylic acid challenge

All patients performed a double-blind, placebo-controlled challenge (DBPC) with ASA. For the DBPC we used ASA and placebo. They were in gelatin capsule (LoFarma, Milan, Italy). None of the patients presented urticaria symptoms at the time of testing. Antihistamines had been interrupted 3 days before the challenges. An informed consent was obtained from all the subjects. Challenges were administered using a double-blind placebo-controlled procedure during the morning hours. Before DBPC, patients received a sham-challenge with placebo (talc). When no urticaria symptoms were noted after placebo, 1 hour later, DBPC was performed using ASA and placebo. They were given in a randomized sequence: three placebo capsules and three doses of ASA (10 mg, 10 mg, and finally 20 mg). Each dose was given after 1 hour, if no symptoms had developed with the previous administration. Only the appearance of unequivocal worsening of urticaria (defined as pruritic and erythematous areas raised over normal skin) and/or the appearance of angioedema (defined as swelling of the skin and/or external mucosa) were considered as a positive response [11–13].

HLA class-I phenotype and HLA-DRB1 genotype*

HLA class-I antigens were typed by serology with human antisera using the standard complement-dependent microlymphocytotoxicity assay, using a panel of 100 lymphocytes. The antisera used for typing locus A were 1, 2, 3, 9, 10, 11, 23, 24, 25, 26, 28, 29, 30, 31, 32, 33, 34, 35, 37, 40, 43, 66, 68, 69, 74; for typing locus B 5, 7, 8, 12, 13, 14, 15, 16, 17, 18, 21, 22, 23, 27, 37, 38, 39, 40, 41, 42, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 60, 61, 62, 63, 64, 65, 70, 71, 72, 73, 75, 76, 77; for typing locus Cw 1, 2, 3, 4, 5, 6, 7, 8, 17.

DNA was prepared from ethylenediamine tetraacetic acid-anticoagulated blood. A salting-out method was used to extract genomic DNA, which was then precipitated, washed with ethanol, and resuspended in water. Then, for each sample, HLA class-II genotyping was performed for HLADRB1*, evaluating the 1, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, and 17 alleles, by using a "low-resolution" polymerase chain reaction amplification with sequence-specific primers (PCR-SSP) (Olerup Low-Resolution Kit).

Statistical methods

Data were analyzed with SPSS 13.0 statistical package (SPSS Inc, Chicago, Ill). Quantitative data were assessed with Student *t* test. Associations between qualitative variables, such as prevalence of HLA phenotypes and genotype, in patients and control groups, were analyzed with $\chi 2$-test or Fisher-exact test when indicated. Statistical significance was set at P values smaller than .05. To better evaluate the potential association between HLA phenotypes and genotype and AICU, we also performed a multiple logistic regression analysis, including all the HLA phenotypes and genotype with a significant different distribution in the univariate analysis. To assess the strength of these associations, odds ratios (OR), with 95% confidence interval (95% CI), were calculated.

RESULTS

The frequency of HLA class-I phenotypes and HLA-DRB1* alleles in patients with AICU and in the control group is shown in Tables 1(a)-1(b).

In our population, 6 HLA class-I antigens presented a significantly different distribution between patients and controls; whereas no significant difference was found for the HLADRB1* alleles distribution. Subjects with HLA-B44

Table 1: (a)–(d): HLA class-I (A, B, and Cw) phenotypes and HLA-DRB1* genotypes in patients affected by AICU and in the control group.

	Control group ($n = 200$)	CIU AICU group ($n = 69$)	P
HLA-A (phenotype carrier)			
1	21.0%	23.2%	NS*
2	48.5%	52.2%	NS
3	18.5%	24.6%	NS
9	0%	0%	NS
10	0.5%	0%	NS
11	**13.0%**	**4.3%**	**.046**
23	6.0%	2.9%	NS
24	32.5%	26.1%	NS
25	2.5%	7.2%	NS
26	8.0%	5.8%	NS
28	0.5%	4.3%	NS
29	2.0%	2.9%	NS
30	7.5%	2.9%	NS
31	6.5%	2.9%	NS
32	8.0%	5.8%	NS
33	4.5%	5.8%	NS
34	0 %	0 %	NS
35	0 %	0 %	NS
37	0 %	0 %	NS
40	0 %	0 %	NS
43	0 %	0 %	NS
66	0.5%	0 %	NS
68	9.5%	11.6%	NS
69	0 %	2.9%	NS
74	0 %	0 %	NS

(a)

HLA-B (phenotype carrier)	Control group ($n = 200$)	CIU AICU group ($n = 69$)	P
5	0%	0 %	NS
7	13.5%	11.6%	NS
8	13.5%	5.8%	NS
12	0%	0%	NS
13	**9.5%**	**0%**	**.005**
14	1.5%	0 %	NS
15	2.0%	0 %	NS
16	0.5%	0 %	NS
17	2.0%	1.4%	NS
18	17.0%	23.2%	NS
21	0 %	0 %	NS
22	0 %	0 %	NS
23	0 %	1.4%	NS
27	6.0%	7.2%	NS
35	29.0%	24.6%	NS
37	3.0%	4.3%	NS
38	4.5%	4.3%	NS
39	8.0%	4.3%	NS
40	2.5%	0 %	NS
41	0.5%	4.3%	NS
42	0 %	0 %	NS
44	**13.0%**	**29.0%**	**0.002**
45	1.0%	1.4%	NS

(b)

HLA-B (phenotype carrier)	Control group ($n = 200$)	CIU AICU group ($n = 69$)	P
46	0%	0%	NS
47	1.0%	1.4%	NS
49	7.5%	7.2%	NS
50	6.0%	1.4%	NS
51	20.5%	23.2%	NS
52	1.0%	0%	NS
53	2.0%	2.9%	NS
54	0 %	0%	NS
55	4.0%	2.9%	NS
56	1.0%	1.4%	NS
57	8.5%	2.9%	NS
58	3.0%	4.3%	NS
60	3.5%	2.9%	NS
61	0.5%	0 %	NS
62	6.0%	8.7%	NS
63	1.0%	1.4%	NS
64	0 %	0 %	NS
65	1.5%	4.3%	NS
70	0 %	0 %	NS
71	0 %	0 %	NS
72	0 %	0 %	NS
73	0.5%	0 %	NS
75	0.5%	0 %	NS
76	0 %	0 %	NS
77	0 %	0 %	NS

(b) Continued.

HLA-Cw (phenotype carrier)	Control group ($n = 200$)	CIU AICU group ($n = 69$)	P
1	9.0%	5.8%	NS
2	8.5%	11.6%	NS
3	16.0%	8.7%	NS
4	**37.0%**	**14.5%**	**.001**
5	**5.5%**	**14.5%**	**.016**
6	17.5%	8.7%	NS
7	**48.5%**	**23.2%**	**< .001**
8	0 %	1.4%	NS
17	1.0%	0 %	NS

(c)

HLA-DRB1* (allele carrier)	Control group ($n = 200$)	CIU AICU group ($n = 69$)	P
1	16.0%	15.9%	NS
3	21.0%	14.5%	NS
4	15.5%	10.1%	NS
7	24.0%	26.1%	NS
8	7.5%	4.3%	NS
9	1.0%	0 %	NS
10	3.5%	0 %	NS
11	39.5%	44.9%	NS
12	6.5%	4.3%	NS
13	22.0%	26.1%	NS
14	7.5%	11.6%	NS
15	15.5%	8.7%	NS
16	12.0%	8.7%	NS
17	1.0%	0 %	NS

(d)

* By $\chi 2$ test or Fisher's exact test; NS: not significant.

antigen were more represented in patients affected by AICU than in control group (29% versus 13%; $P = .002$), as well as subjects with HLA-Cw5 antigen (14.5% versus 5.5%; $P = .016$). On the other hand, subjects with HLA-A11 (4.3% versus 13%; $P = .046$), HLA-B13 (0% versus 9.5%; $P = .005$), HLA-Cw4 (14.5% versus 37%; $P = .001$), and HLA-Cw7 antigen (23.2% versus 48.5%; $P < .001$) were more represented in control group than in patients affected by AICU. Then we performed a multiple-logistic regression, including all the above-mentioned HLA antigens; only three HLA phenotypes maintained a significant different distribution, more precisely, HLA-Cw4 (OR 0.18 with 95% CI 0.08–0.40; $P < .001$) and HLA-Cw7 (OR 0.21 with 95% CI 0.11–0.42; $P < .001$) remained associated with a lower risk of AICU, whereas carriers of HLA-B44 phenotype had a higher risk of AICU (OR 2.63 with 95% CI 1.13–6.12; $P = .024$).

DISCUSSION

In this study we demonstrated an association between some HLA class-I antigens and chronic idiopathic urticaria associated with aspirin and/or NSAIDs hypersensitivity (AICU). In particular, two HLA-Cw phenotypes, HLA-Cw4 and HLA-Cw7, seem to be less frequent in patients with AICU, whereas HLA-B44 shows a positive association with this subtype of chronic urticaria. To the best of our knowledge, this is the first description of such association.

Many studies have investigated the etiology of chronic urticaria. Recent researches have focused primarily on the demonstration of IgG antibodies directed to the α subunit of the high affinity IgE receptor (FcεRI) on mast cell and basophils, revealed by autologous serum skin test, and on the copresence of aspirin and/or NSAIDs and/or food additives hypersensitivity aggravating urticaria. However, in many patients, the exact mechanisms governing regional mast cell or basophil activation are unknown; this condition is usually indicated by the term of "ordinary chronic idiopathic urticaria" [1, 2].

Besides, many questions still remain to be answered, such as the triggering of the autoreactive process, other immunological or not immunological pathogenetic mechanisms, temporary spontaneous remissions, and genetic heterogeneity of the patients [3–5].

gKim et al suggested that some HLA class-II genotypes are a strong genetic marker to determine the aspirin-induced urticaria phenotype [14]. However, the data have not been confirmed by other studies [15, 16]. The HLA class-I loci, at this time, are relatively less investigated than HLA class-II loci in reference to disease predisposition. Therefore, HLA class-I antigen plays a major role in ankylosing spondylitis [9]. Nevertheless, it is interesting to emphasize that some HLA class-I alleles have been reported to be associated with adverse events in patients taking various drugs: HLA-A29 and B12 with Stevens-Johnson syndrome induced by sulphonamide, HLA-A2 and B12 with Stevens-Johnson syndrome induced by NSAIDs [17, 18]. Recently, the HLA-B*5801 allele has been proposed as a genetic marker for severe cutaneous adverse reactions caused by allopurinol [19].

Furthermore and interestingly, new functions, that could play a role in pathogenetic processes, have been identified for HLA class-I molecules, mainly for HLA-Cw, such as the inhibition of the lytic capacity of natural killer cells and non-MHC restricted T cells [20].

It is now tempting to speculate that some of HLA class-I molecules could be involved in some of the heterogeneous pathogenetic mechanisms of AICU and, consequently, that HLA variants could induce this subtype of chronic urticaria.

In conclusion, we assume that HLA alleles may be involved in the development of AICU as possible predisposing factors.

ABBREVIATIONS

HLA	human leucocyte antigen
NSAIDs	nonsteroidal anti-inflammatory drugs
AICU	chronic idiopathic urticaria associated with aspirin and/or NSAIDs hypersensitivity

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