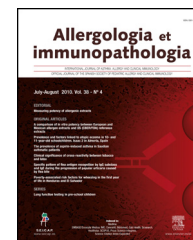


Allergologia et immunopathologia

www.elsevier.es/ai



ORIGINAL ARTICLE

CTLA-4 polymorphism in the pathogenesis of chronic spontaneous autoreactive urticaria[☆]

Z. Brzoza^{a,*}, W. Grzeszczak^b, B. Rogala^a, W. Trautsolt^b, D. Moczulski^c

^a Chair and Clinical Department of Internal Diseases, Allergology and Clinical Immunology, Medical University of Silesia, Katowice, Poland

^b Chair and Clinical Department of Internal Diseases, Diabetology and Nephrology, Medical University of Silesia, Katowice, Poland

^c Chair and Clinical Department of Internal Diseases and Nephrodiabetology, Medical University of Łódź, Łódź, Poland

Received 25 September 2012; accepted 2 January 2013

KEYWORDS

Autoimmunity;
Chronic urticaria;
CTLA-4;
Polymorphism;
Thyroid

Abstract

Background: Autoimmune mechanisms are considered to play a significant role in chronic urticaria pathophysiology. Additionally, clinical experience emphasises the coexistence of chronic urticaria manifestation with thyroid autoimmunity. As the role of CTLA-4 polymorphism in autoimmune thyroid diseases is well proven we speculated on the possible role of this polymorphism in the background of chronic urticaria.

Materials and methods: We included 128 chronic spontaneous autoreactive urticaria patients (87 females and 41 males) and 101 healthy volunteers (71 females and 30 males). In all examined subjects CTLA-4 A49G polymorphism was analysed. Disease severity with Urticaria Activity Score as well as age of disease onset was also studied.

Results: No statistically significant differences in the allele or genotype distribution between urticaria patients and controls were observed. Furthermore, we found no association between CTLA4 polymorphism and urticaria severity as well as the age of disease onset.

Conclusions: Our data suggest that there is no contribution of CTLA-4 A49G polymorphism to chronic spontaneous autoreactive urticaria susceptibility. We recommend further research on other polymorphisms in chronic urticaria patients to explore in detail the potent role of the genetic background in the pathogenesis of this disorder.

© 2012 SEICAP. Published by Elsevier España, S.L. All rights reserved.

Introduction

Chronic urticaria (CU) is a debilitating skin disorder with poorly understood pathogenesis. Autoimmune origin should be considered in CU pathophysiology. Strong dependency between autoimmune CU and major histocompatibility

[☆] Study was supported by a research Grant from the Medical University of Silesia.

* Corresponding author.

E-mail address: zbrzoza@mp.pl (Z. Brzoza).

complex allele suggests the role of genetic background in the pathogenesis of urticaria and indicates the association to other autoimmune diseases. Clinical experience emphasises the coexistence of CU with thyroid autoimmunity. Furthermore, autoimmune thyroid diseases (AITDs) can often manifest in association with other autoimmune disorders such as diabetes, vitiligo, multiple sclerosis, Addison's disease, rheumatoid arthritis and systemic lupus erythematosus. AITD is supposed to be a polygenic pathology with no major predisposition locus. The cytotoxic T-lymphocyte associated antigen 4 (*CTLA-4*) gene has been proven to be a susceptibility factor, separate from the *HLA* locus, in Graves' disease and Hashimoto thyroiditis.^{1–3} The *CTLA4* region and the *HLA* locus have been proposed as the loci that predispose to AITDs with an independent impact.⁴

CTLA4 is a regulatory molecule expressed on the surface of activated T lymphocytes providing a negative signal to the T cell, which limits immune responses.^{5–7} *CTLA4* induces antigen-specific apoptosis and limits T lymphocyte proliferation.^{6,8} In this mechanism it plays a role in regulating and maintaining self-tolerance. Human *CTLA4* gene consists of four exons and three introns and its association with AITDs is suspected in relation to adenine to guanine transition at position 49 of exon 1 (A49G).⁹ This single nucleotide polymorphism results in the substitution of threonine with alanine at codon 17 of the leader sequence. Bearing in mind the relationship of the G49 allele to a weaker suppressing function of the Ala17 molecular variant of *CTLA4*, G/G homozygous patients potentially have a greater risk of AITD compared to the A/A genotype.

In our previous studies we proved the role of another autoimmunity related gene, namely *PTPN22* in chronic spontaneous urticaria pathogenesis, meanwhile *PDCD1* appeared not to be involved.^{10–12} The aim of the present study is to test the possible role of the *CTLA4* gene in the susceptibility to CU.

Materials and methods

Characteristics of study sample

The study group consisted of 128 unrelated CU patients (87 females and 41 males, mean age 38.8 years, aged 20–60 years) with positive autologous serum skin test (ASST) result,

without concomitant thyroid pathology. Precise medical history and physical examination resulted in establishing the diagnosis of spontaneous urticaria without known eliciting factors. The control group was composed of 101 unrelated healthy volunteers (71 females and 30 males, mean age 44.2 years, aged 19–59 years). All individuals were Caucasians and came from the Polish population.

Urticaria Activity Score (UAS) was used to detect disease severity. This symptom assessment tool analyses wheals and pruritus intensity.^{13,14} As recommended, we performed a seven-day assessment (UAS7) and yielded a total score of 0–42. All patients were treated according to current guidelines.¹⁵ The age of disease onset was also analysed.

Genomic DNA isolation and *CTLA-4* genotyping

Genomic DNA from blood leukocytes was obtained using MasterPure™ DNA Purification Kit (Epicentre Technologies, Madison, Wisconsin, USA), in accordance with the manufacturer's indications. In all examined subjects *CTLA-4* A49G polymorphism was analysed. Genotyping by allelic discrimination using Custom TaqMan SNP Genotyping Assays and 7300 Real-time PCR System (Applied Biosystems, Foster City, CA, USA) was performed.

Statistical analysis

The differences in allele and genotype frequencies between groups were calculated with Chi-square test. Additionally, odds ratios (OR) with 95% confidence intervals (CI) were estimated. ANOVA test was used for UAS7 and age of disease onset comparisons between different genotype division subgroups (Statistica 8.0 PL, Statsoft Inc., USA).

The study was approved by the Bioethics Committee of the Medical University of Silesia in Katowice.

Results

The assay for Hardy–Weinberg equilibrium revealed no deviation in both analysed groups. In the allele or genotype distribution no statistically significant differences between CU patients and controls were found (Table 1). Furthermore, we found no association between *CTLA4* and urticaria severity as well as the age of disease onset when analysed in different genotype division subgroups (Table 2).

Table 1 *CTLA4* A49G genotype and allele distribution in chronic urticaria (CU) patients and healthy controls.

SNP	Group	Genotype total number (%)			Allele total number (%)		OR (95% CI)	<i>p</i>
A49G	CU	AA	AG	GG	A	G	0.969 (0.537–1.741)	0.978
		44 (34)	65 (51)	19 (15)	153 (60)	103 (40)		
	Controls	AA	AG	GG	A	G	<i>p</i> ' = 0.947	
		34 (34)	53 (52)	14 (14)	121 (60)	81 (40)		

SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval; *p*: statistical significance (*p*': allele frequency statistical significance); the odds ratio was calculated for patients homozygous or heterozygous carrying risk allele vs. homozygous.

Table 2 Urticaria Activity Score one-week assessment (UAS7) and age of disease onset in chronic urticaria patients with A49G different genotypes.

	Genotype distribution			<i>p</i>
	AA	AG	GG	
UAS7 (points)	20.4 ± 8.03	21.7 ± 8.42	19.5 ± 7.59	ns
Age of disease onset (years)	37.3 ± 7.83	34.6 ± 6.79	36.1 ± 7.17	ns

p: statistical significance; ns: statistically non-significant; data are presented as mean ± standard deviation.

Discussion

Autoimmune diseases share a common set of susceptibility genes. AITDs are among the most common autoimmune conditions. *CTLA4* gene is related to autoimmunity in general without organ-specificity, and its role in AITDs is rather non-specific.^{16–18} The relationship between the *CTLA4* variation and the downregulation of T cell activation is well proven.^{19,20} Nucleotide variation, which is an adenine to guanine transition, occurs at location 49 (A49 G) of exon 1 and results in amino acid substitution of alanine to threonine of codon 17 of the leader peptide.²¹ The exon 1 polymorphism markedly influences an inhibitory function of *CTLA4*. G49 allele of codon 17 dimorphism is suspected to be related to the reduced control of T cell proliferation and was proven to be associated with the overproduction of autoantibodies, including production of thyroglobulin and thyroid peroxidase autoantibodies.^{19,22,23}

Besides *HLA*, *CTLA4* is the only locus for which dependency with Graves' disease has been presented in different populations. Interestingly, the area of relation to Graves' disease on chromosome 2q31–q33 is close to the region of susceptibility to insulin-dependent diabetes mellitus.^{21,24} Additionally, the association between this locus and other autoimmune diseases such as multiple sclerosis, Addison's disease, rheumatoid arthritis and systemic lupus erythematosus was presented.²⁵ Taken together, *CTLA4* is proposed to be the *HLA*-independent locus of human autoimmunity.

Because of the frequent coexistence of CU and AITDs as well as proven genetic susceptibility to AITDs, we hypothesised CU genetic pathogenesis. Bearing in mind that *CTLA4* polymorphism is generally connected with the production of autoantibodies, we selected patients with positive ASST as manifesting autoreactive variant of CU. In our study no statistically significant differences in the *CTLA4* allele and genotype distribution between CU patients and controls were found. This finding is interesting in the light of the frequent coexistence of CU and Hashimoto thyroiditis. Undoubtedly, polymorphisms of the *CTLA4* gene show strong association with Graves' disease whereas the findings in Hashimoto thyroiditis are contrary. Additionally, A49G dimorphism allele frequencies can vary in relation to ethnic differences. Although A49 allele dominates in the Caucasian population, whereas G49 allele is more frequent in Asian population,²⁶ our study group was ethnically homogenous. Altogether the study data suggest no contribution of this polymorphism to CU susceptibility.

Autoimmune disorder is an effect of genetic susceptibility loci cooperation, although those loci independently present a rather small risk of defect. In the chromosome

2q33–34 there is a cluster of homologous genes (*CTLA4*, *CD28* and *ICOS*) related to immune activation and considered promising candidate genes for susceptibility to autoimmune diseases.²⁷ We conclude that further genetic studies on other polymorphisms and other loci in this area in CU patients are necessary to explore deeply the potent role of the genetic background in its pathogenesis.

Ethical disclosures

Patients' data protection. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appears in this article.

Protection of human subjects and animals in research. The authors declare that the procedures followed were in accordance with the regulations of the responsible Clinical Research Ethics Committee and in accordance with those of the World Medical Association and the Helsinki Declaration.

Conflict of interest

Authors declare no conflict of interest.

References

1. Donner H, Braun J, Seidl C, Rau H, Finke R, Ventz M, et al. Codon 17 polymorphism of the cytotoxic T lymphocyte antigen 4 gene in Hashimoto's thyroiditis and Addison's disease. *J Clin Endocrinol Metab.* 1997;82:4130–2.
2. Donner H, Rau H, Walfish PG, Braun J, Siegmund T, Finke R, et al. Badenhoop K 1997b CTLA4 alanine-17 confers genetic susceptibility to Graves' disease and to type 1 diabetes mellitus. *J Clin Endocrinol Metab.* 1997;82:143–6.
3. Yanagawa T, Hidaka Y, Guimaraes V, Soliman M, DeGroot LJ. CTLA-4 gene polymorphism associated with Graves' disease in a Caucasian population. *J Clin Endocrinol Metab.* 1995;80:41–5.
4. Tomer Y, Davies TF. The genetic susceptibility to Graves' disease. *Baillieres Clin Endocrinol Metab.* 1997;11:431–50.
5. Chambers CA, Allison JP. Co-stimulation in T cell responses. *Curr Opin Immunol.* 1997;9:396–404.
6. Gribben JG, Freeman GJ, Boussiotis VA, Rennert P, Jellis CL, Greenfield E, et al. CTLA4 mediates antigenspecific apoptosis of human T cells. *Proc Natl Acad Sci USA.* 1995;92:811–5.
7. Scheipers P, Reiser H. Fas-independent death of activated CD4 T lymphocytes induced by CTLA-4 crosslinking. *Proc Natl Acad Sci USA.* 1998;95:10083–8.
8. Bugeon L, Dallman MJ. Costimulation of T cells. *Am J Respir Crit Care Med.* 2000;162:5164–8.

9. Dariavach P, Mattei MG, Golstein P, Lefranc MP. Human Ig superfamily CTLA-4 gene: chromosomal localization and identity of protein sequence between murine and human CTLA-4 cytoplasmic domains. *Eur J Immunol.* 1998;18:1901–5.
10. Brzoza Z, Grzeszczak W, Trautsolt W, Moczulski D. Protein tyrosine phosphatase-22 (PTPN-22) polymorphism in the pathogenesis of chronic urticaria. *Allergy.* 2011;66:1392–3.
11. Brzoza Z, Grzeszczak W, Rogala B, Trautsolt W, Moczulski D. PTPN22 polymorphism presumably plays a role in the genetic background of chronic spontaneous autoreactive urticaria. *Dermatology.* 2012;224:340–5.
12. Brzoza Z, Grzeszczak W, Trautsolt W, Moczulski D. Lack of association of programmed cell death 1 gene (PDCD1) polymorphisms with susceptibility to chronic urticaria in patients with positive autologous serum skin test. *J Investig Allergol Clin Immunol.* 2012;22:432–6.
13. Młynek A, Zalewska-Janowska A, Martus P, Staubach P, Zuberbier T, Maurer M. How to assess disease activity in patients with chronic urticaria? *Allergy.* 2008;63:777–80.
14. Zuberbier T, Asero R, Bindslev-Jensen C, Walter Canonica G, Church MK, Giménez-Arnau A, et al. EAACI/GA[2]LEN/EDF/WAO guideline: definition, classification and diagnosis of urticaria. *Allergy.* 2009;64:1417–26.
15. Zuberbier T, Asero R, Bindslev-Jensen C, Walter Canonica G, Church MK, Giménez-Arnau A, et al. EAACI/GA[2]LEN/EDF/WAO guideline: management of urticaria. *Allergy.* 2009;64:1427–43.
16. Einarsdottir E, Soderstrom I, Lofgren-Burstrom A, Haraldsson S, Nilsson-Ardnor S, Penha-Goncalves C, et al. The CTLA4 region as a general autoimmunity factor: an extended pedigree provides evidence for synergy with the HLA locus in the etiology of type 1 diabetes mellitus, Hashimoto's thyroiditis and Graves' disease. *Eur J Hum Genet.* 2003;11:81–4.
17. Rapoport B, McLachlan SM. Thyroid autoimmunity. *J Clin Invest.* 2001;108:1253–9.
18. Weetman AP. Autoimmune thyroid disease: propagation and progression. *Eur J Endocrinol.* 2003;148:1–9.
19. Kouki T, Sawai Y, Gardine CA, Fisfalen ME, Alegre ML, DeGroot LJ. CTLA-4 gene polymorphism at position 49 in exon 1 reduces the inhibitory function of CTLA-4 and contributes to the pathogenesis of Graves' disease. *J Immunol.* 2000;165:6606–11.
20. Maurer M, Loserth S, Kolb-Maurer A, Ponath A, Wiese S, Kruse N, et al. A polymorphism in the human cytotoxic T-lymphocyte antigen 4 (CTLA4) gene (exon 1+49) alters T-cell activation. *Immunogenetics.* 2002;54:1–8.
21. Nistico L, Buzzetti R, Pritchard LE, Van der Auwera B, Giovannini C, Bosi E, et al. The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. *Hum Mol Genet.* 1996;5:1075–80.
22. Tomer Y, Greenberg DA, Barbesino G, Concepcion E, Davies TF. CTLA-4 and not CD28 is a susceptibility gene for thyroid autoantibody production. *J Clin Endocrinol Metab.* 2001;86:1687–93.
23. Zaletel K, Krhin B, Gaberscek S, Pirnat E, Hojker S. The influence of the exon 1 polymorphism of the cytotoxic T lymphocyte antigen 4 gene on thyroid antibody production in patients with newly diagnosed Graves' disease. *Thyroid.* 2002;12:373–6.
24. Vaidya B, Imrie H, Perros P, Young ET, Kelly WF, Carr D, et al. The cytotoxic T lymphocyte antigen-4 is a major Graves' disease locus. *Hum Mol Genet.* 1999;8:1195–9.
25. Kristiansen OP, Larsen ZM, Pociot F. CTLA-4 in autoimmune diseases – a common susceptibility gene to autoimmunity? *Genes Immun.* 2000;1:170–84.
26. Yung E, Cheng PS, Fok TF, Wong GW. CTLA-4 gene A-G polymorphism and childhood Graves' disease. *Clin Endocrinol (Oxf).* 2002;56:649–53.
27. Marron MP, Zeidler A, Raffel LJ, Eckenrode SE, Yang JJ, Hopkins DI, et al. Genetic and physical mapping of a type 1 diabetes susceptibility gene (IDDM12) to a 100-kb phagemid artificial chromosome clone containing D2S72-CTLA4-D2S105 on chromosome 2q33. *Diabetes.* 2000;49:492–9.