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ORIGINAL ARTICLE

HLA-C*17, DQB1*03:01, DQA1*01:03 and DQA1*05:05 Alleles Associated to Bullous Pemphigoid in Brazilian Population

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Background: Bullous pemphigoid (BP) is an autoimmune disease with bullous vesicles and an incidence of 0.2 to 1.4 per 100,000 inhabitants. Many studies have been published demonstrating the association of pemphigoid with HLA class II system alleles in different populations, however there are no data on the BP, one of the most heterogeneous in the world. **Objective:** To typify HLA alleles in Brazilians with Bullous pemphigoid. **Methods:** The study group included 17 Brazilian patients with a confirmed diagnosis of BP from a hospital in Sao Paulo city, southeast Brazil. DNA was extracted from peripheral blood using Qiagen kits and HLA A, B, C, DR and DQ typing was performed using polymerase chain reaction. The control group was composed of a database of 297 deceased donors from the city of Sao Paulo. The statistical significance level was adjusted using the Bonferroni correction depending on the phenotypic frequencies evaluated for HLA class I (A, B and C) and class II (DRB1, DQB1 and DQA1). **Results:** Our findings show that alleles HLA C*17, DQB1*03:01, DQA1*01:03 and DQA1*05:05 are associated with the onset of the disease in the Brazilian population, with relative risks of 8.31 (2.46 to 28.16), 3.76 (1.81 to 7.79), 3.57 (1.53 to 8.33), and 4.02 (1.87 to 8.64), respectively ($p<0.005$). **Conclusion:** Our data indicate that Brazilian patients with BP present the same genetic predisposition linked to HLA-DQB1*03:01 previously reported in Caucasian and Iranian individuals and our study introduces three new alleles (C*17, DQA1*01:03 and DQA1*05:05) involved in the pathophysiology of BP. **(Ann Dermatol 30(1) 8～12, 2018)**

-Keywords-
Genes, MHC class I, Genes, MHC class II, HLA antigens, Pemphigoid, bullous, Polymerase chain reaction

INTRODUCTION

Bullous pemphigoid (BP) is an autoimmune disease characterized by subepidermal blistering occurring mainly in elderly people between 60 and 70 years of age[1]. Its annual incidence is estimated at between 0.2 and 1.4 per 100,000 person-years[2-4], and this value will probably increase with the increasing proportion of the elderly in the population. The pathophysiology is characterized by the action of autoantibodies against specific antigens in hemidesmosomes at the epidermal junction[5] causing separation of the epidermis from the dermis.

Thus, the immune response of BP is characterized by the action of circulating autoantibodies of immunoglobulin (Ig)G and C3 against the structural components of the basement membrane zone (BMZ)[6,7]. These autoantibodies have two main targets: antigen 1 (BPAG1, also known as BP230) and antigen 2 (BPAG2, BP180 or collagen type

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XVII). It is likely that this autoimmunity involves autoreactive T cells that recognize epitopes located in the extracellular region of BP180, mainly in the NC16A ectodomain[8,9].

There is strong evidence that genetic factors including HLA alleles may promote activation of potentially autoreactive T cells[10-12] and different alleles and haplotypes have been identified in different populations.

Caucasian patients with BP were the first to be associated with the allele HLA-DQB1*03:01[13], and it was later associated with Iranian patients[14]. However, in the Japanese population, patients with BP were associated with haplotypes HLA-DRB1*04/DQA1*03:01/DQB1*03:02 and DRB1*11:01/DQA1*0505/DQB1*03:02, and individual alleles DRB1*11:01 and DQB1*03:02[15].

However, in the Chinese population with BP[16], there was no statistically significant association with DQB1*03:01, but there was a protective factor for BP in individuals who presented the DRB1*08 *loci* (DR8) and DRB1*08/DQB1*06.

These findings indicate that different HLA class II alleles and haplotypes can influence the genetic susceptibility to BP in different populations.

Therefore, this study was conducted to evaluate the association of HLA alleles (*loci* A, B, C, DR and DQ) with BP in Brazilian patients, by comparing the prevalence of these alleles with those in a control group.

MATERIALS AND METHODS

Patients and controls

For the prospective case–control study, 17 consecutive patients (eight male and nine female; age 63.71 years, range 20～71 years) diagnosed with BP were selected to participate. All patients attended the Department of Otolaryngology of Sao Paulo University Hospital, Brazil. After explanation of the purpose of the research, the participants read and signed the consent forms, and were included in the survey. All patients were diagnosed according to clinical features, histopathological analysis after biopsy, and direct or indirect immunofluorescence showing IgG/C3 complement at BMZ. A "salt split test" confirmed all of the cases. The control group consisted of 297 (114 female and 183 male; age 43±17 years) deceased organ donors (Table 1) from Sao Paulo City, Sao Paulo, Brazil who died between 1 January 2011 and 22 March 2012.

The local ethics committee (CAPPesq) approved this study (IRB no. 17.201).

DNA extraction and HLA typing

DNA was extracted from peripheral blood using Qiagen kits (QIAamp DNA Mini Kit®; Qiagen, Valencia, CA, USA). HLA A, B, C, DRB1, DQA1 and DQB1 typing was performed using polymerase chain reaction and amplification using sequence-specific oligonucleotide contained in LABType Kits® (One Lambda Inc., Canoga Park, CA, USA).

Statistical analysis

The relationship between the frequencies of HLA and the presence of BP was measured with the odds ratio (OR), using logistic regression, and with a 95% confidence interval (CI) to the calculated OR. The statistical tests were ad-

Table 1. Prevalence of phenotypic alleles of HLA C for patients with pemphigoid and organ donors, HCFMUSP, 2016

HLA C	Pemphigoid (n=34)	Control (n=592)	OR (95% CI)	*p*-value*,†
C*01	0	14 (2.4)	NA	NA
C*02	1 (2.9)	33 (5.6)	0.55 (0.07～4.13)	0.558
C*03	2 (5.9)	70 (11.8)	0.50 (0.12～2.13)	0.346
C*04	4 (11.8)	99 (16.7)	0.71 (0.24～2.07)	0.533
C*05	5 (14.7)	31 (5.2)	3.35 (1.21～9.30)	0.020
C*06	2 (5.9)	60 (10.1)	0.59 (0.14～2.54)	0.479
C*07	9 (26.5)	111 (18.8)	1.70 (0.76～3.77)	0.195
C*08	1 (2.9)	44 (7.4)	0.40 (0.05～3.01)	0.375
C*12	4 (11.8)	37 (6.3)	2.14 (0.71～6.43)	0.174
C*14	0	20 (3.4)	NA	NA
C*15	0	26 (4.4)	NA	NA
C*16	0	29 (4.9)	NA	NA
C*17	4 (11.8)	10 (1.7)	8.31 (2.46～28.16)	0.001‡
C*18	0	8 (1.4)	NA	NA

Values are presented as number of alleles (%). HCFMUSP: Faculdade de Medicina da Universidade de Sao Paulo (Faculty of Medicine of the University of Sao Paulo), OR: odds ratio, CI: confidence interval, NA: not evaluable. *p-value using logistic regression. †Significance level with Bonferroni correction for $\alpha=0.05$: $\alpha/14=0.05/14=0.0035714$. ‡Significance $p<0.0035714$.

justed using the Bonferroni correction depending on the number of alleles present. All data were stored and analyzed with two statistical software packages: PASW Statistics for Windows ver. 18.0 (IBM Co., Armonk, NY, USA) and Stata ver. 11.0. Significance level with Bonferroni correction for $\alpha = 0.05$: $\alpha/14 = 0.05/14 = 0.004$. *Significant $p < 0.0035714$.

RESULTS

Of the 17 cases (eight male and nine female) with pemphigoid, only one cited a reaction following the use of amoxicillin and one after a major "stress" crisis. The group covered four of the five Brazilian macro-regions and the average age of the patients was 63.71 years (range, 20～71 years) with 10 white patients (58.8%), 6 "mulatto" (35.3%), and only 1 black patient (5.9%).

HLA class I

There was no significant association between the frequencies of HLA A or HLA B and BP. However, HLA C analysis found an association with HLA C*17 (Table 1). Individuals with HLA C*17 had eight times (11.8% vs. 1.7%; $p=0.001$; OR, 8.31; 95% CI, 2.46～28.16) greater chance of having the disease compared with the control group.

HLA class II

There was no significant association between the frequencies of HLA DRB1 and BP. However, the presence of DQB1*03:01 (38.2% vs. 14.1%; $p=0.000372$; OR, 3.76; 95% CI, 1.81～7.79), DQA1*01:03 (23.5% vs. 7.9%; $p=0.003$; OR, 3.57; 95% CI, 1.53～8.33), and DQA1*05:05 (32.4% vs. 10.8%; $p=0.000358$; OR, 4.02; 95% CI, 1.87～8.64) (Table 2, 3) was significantly higher in BP patients compared to those who did not have these alleles.

DISCUSSION

BP is an autoimmune-mediated blistering disease affecting the elderly. It is assumed that autoreactive T cell responses to BPAG2 are elicited upon recognition of this antigen bound to the HLA class II region of DQB1 molecule[17,18].

Table 2. Prevalence of phenotypic alleles of HLA DQB1 for patients with pemphigoid and organ donors, HCFMUSP, 2016

HLA DQB1	Pemphigoid (n=34)	Control (n=594)	OR (95% CI)	*p*-value*,†
DQB1*02:01	0	59 (9.9)	NA	NA
DQB1*02:02	2 (5.9)	74 (12.5)	0.44 (0.10～1.87)	0.266
DQB1*02:03	0	1 (0.2)	NA	NA
DQB1*03:01	13 (38.2)	84 (14.1)	3.76 (1.81～7.79)	0.000372‡
DQB1*03:02	1 (2.9)	52 (8.8)	0.32 (0.04～2.36)	0.261
DQB1*03:03	1 (2.9)	9 (1.5)	1.97 (0.24～16.01)	0.526
DQB1*03:04	0	1 (0.2)	NA	NA
DQB1*03:19	0	13 (2.2)	NA	NA
DQB1*04:01	0	2 (0.3)	NA	NA
DQB1*04:02	4 (11.8)	42 (7.1)	1.75 (0.59～5.21)	0.313
DQB1*04:04	0	2 (0.3)	NA	NA
DQB1*05:01	3 (8.8)	83 (14.0)	0.60 (0.18～1.99)	0.401
DQB1*05:02	1 (2.9)	18 (3.0)	0.97 (0.13～7.49)	0.976
DQB1*05:03	0	21 (3.5)	NA	NA
DQB1*05:07	0	2 (0.3)	NA	NA
DQB1*06:01	0	5 (0.8)	NA	NA
DQB1*06:02	5 (14.7)	59 (9.9)	1.56 (0.58～4.19)	0.375
DQB1*06:03	3 (8.8)	38 (6.4)	1.42 (0.41～4.84)	0.579
DQB1*06:04	1 (2.9)	18 (3.0)	0.97 (013～7.49)	0.976
DQB1*06:05	0	1 (0.2)	NA	NA
DQB1*06:08	0	1 (0.2)	NA	NA
DQB1*06:09	0	3 (0.5)	NA	NA
DQB1*06:11	0	4 (0.7)	NA	NA
DQB1*06:19	0	1 (0.2)	NA	NA
DQB1*06:27	0	1 (0.2)	NA	NA

Values are presented as number of alleles (%). HCFMUSP: Faculdade de Medicina da Universidade de Sao Paulo (Faculty of Medicine of the University of Sao Paulo), OR: odds ratio, CI: confidence interval, NA: not evaluable. *p-value using logistic regression. †Significance level with Bonferroni correction for $\alpha=0.05$: $\alpha/10=0.05/10=0.005$ or $\alpha=0.01$: $\alpha/10=0.01/10=0.001$. ‡Significant $p<0.001$.

Table 3. Prevalence of phenotypic alleles of HLA DQA1 for patients with pemphigoid and organ donors, HCFMUSP, 2016

HLA DQA1	Pemphigoid (n=34)	Control (n=594)	OR (95% CI)	*p*-value*,†
DQA1*01:01	3 (8.8)	80 (13.5)	0.62 (0.19∼2.08)	0.439
DQA1*01:02	2 (5.9)	127 (21.4)	0.23 (0.05∼0.97)	0.045
DQA1*01:03	8 (23.5)	47 (7.9)	3.57 (1.53∼8.33)	0.003‡
DQA1*02:01	1 (2.9)	74 (12.5)	0.21 (0.03∼1.58)	0.130
DQA1*03:01	3 (8.8)	59 (9.9)	0.88 (0.26∼2.95)	0.831
DQA1*03:02	1 (2.9)	20 (3.4)	0.87 (0.11∼6.67)	0.892
DQA1*04:01	3 (8.8)	40 (6.7)	1.34 (0.39∼4.57)	0.642
DQA1*04:02	0	1 (0.2)	NA	NA
DQA1*05:01	0	68 (11.4)	NA	NA
DQA1*05:03	2 (5.9)	10 (1.7)	3.64 (0.77∼17.33)	0.104
DQA1*05:05	11 (32.4)	64 (10.8)	4.02 (1.87∼8.64)	0.000358‡
DQA1*06:01	0	3 (0.5)	NA	NA

Values are presented as number of alleles (%). HCFMUSP: Faculdade de Medicina da Universidade de Sao Paulo (Faculty of Medicine of the University of Sao Paulo), OR: odds ratio, CI: confidence interval, NA: not evaluable. *p-value using logistic regression. †Significance level with Bonferroni correction for $\alpha=0.05$: $\alpha/9=0.05/9=0.0056$ or $\alpha=0.01$: $\alpha/9=0.01/9=0.0011$. ‡Significant $p<0.0011$.

This study reports the association of HLA class I (*loci* A, B and C) and HLA class II (*loci* DR, DQ) with BP, in Sao Paulo city, southeastern Brazil.

The Brazilian population, one of the most heterogeneous in the world, favors case–control studies with genotyping by allowing a reduction in connection imbalance and thus decreasing the chance of false positives[19].

In the present study, most patients had no association with any external factors: only 1 (5.9%) reported a reaction following the use of amoxicillin and 1 (5.9%) reported a period of major stress preceding the symptoms.

In our study, individuals with HLA C*17 are at approximately eight times higher risk of presenting pemphigoid compared to individuals who do not have this allele ($p<0.001$). Although previous studies[13,15] did not report an association of BP with class I alleles in other populations, this study has shown an association in Brazilian patients with BP, and this is the only statistically significant HLA class I reported so far.

However, with respect to HLA class II, in Japanese patients, Okazaki et al.[15] demonstrated that, compared with a control group, patients with BP were associated with haplotypes HLA-DRB1*04/DQA1*03:01/DQB1*03:02 (39.1% vs. 11.7%; $p<0.001$) and DRB1*11:01/DQA1*05:05/DQB1*03:02 (8.7% vs. 0%; $p<0.0001$), and alleles DQB1*03:02 (43.5% vs. 17.2%; $p<0.002$) and DRB1*11:01 (17.4% vs. 3.2%; $p<0.001$). Similarly, in our study, the presence of the allele DQA1*05:05 increased the risk of manifest BP approximately fourfold ($p=0.000358$), however, with respect to other Class II genes reported in the study by Okazaki et al.[15], there were no statistically significant differences.

Furthermore, in our study, the allele DQA1*01:03 showed a relative risk approximately 3.57 times ($p=0.003$) higher in Brazilian individuals with BP. This new association has not been described in the literature yet, thus proving the existence of different alleles in the genesis of pemphigoid, according to the population studied.

Regarding the allele DQB1*03:01, the study by Delgado et al.[13] showed a significant association in the Caucasian population (35.7% vs. 16.05%, $p=0.005$) in all clinical variants of pemphigoid (BP, oral pemphigoid and ocular cicatricial pemphigoid). It is currently the most common allele present in the different populations studied. Similarly, in our study, this allele also showed an approximately 3.76 times greater risk compared to the control group (38.2% vs. 14.1%, $p=0.000372$), demonstrating an even more important role in the genesis of this pemphigoid allele.

Just as in the Caucasian population, another study by Esmaili et al.[14] in Iranian patients with BP found a significant association with the HLA-DQB1*03:01 allele (36% vs. 23.6%, $p=0.02$), the HLA-DQA1*05:01 allele (45% vs. 33%, $p=0.03$) and the HLA-DQB1*04:01 allele (4% vs. 1.6%, $p=0.04$) compared with the control group. However, in our study, we found no association with the HLA-DQA1*05:01 and HLA-DQB1*04:01 alleles in BP patients, so HLA-DQB1*03:01 remains the only common allele among Brazilian and Iranian patients.

On the other hand, in a study in Chinese patients[16], there was a protective factor for BP in individuals who presented the DRB1*08 and DQB1*06 alleles (8.04% vs. 15.19% and 1.54% vs. 13.82%, respectively; $p<0.05$) compared with controls. However, in our study, we did not find a statistically significant difference in DRB1*08 (2.9% vs. 6.7%, $p=0.398$) and DQB1*06 (26.5% vs. 22.1%,

p=0.548) alleles compared with controls in Brazilian patients with BP.

In conclusion, our study demonstrates that alleles HLA C*17, DQB1*03:01, DQA1*01:03, and DQA1*05:05 are associated with BP in Brazilian patients with relative risks of 8.31 (2.46 to 28.16), 3.76 (1.81 to 7.79), 3.57 (1.53 to 8.33), and 4.02 (1.87 to 8.64), respectively.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

REFERENCES

1. Walsh SR, Hogg D, Mydlarski PR. Bullous pemphigoid: from bench to bedside. Drugs 2005;65:905-926.
2. Bernard P, Vaillant L, Labeille B, Bedane C, Arbeille B, Denoeux JP, et al. Incidence and distribution of subepidermal autoimmune bullous skin diseases in three French regions. Bullous Diseases French Study Group. Arch Dermatol 1995;131:48-52.
3. Langan SM, Smeeth L, Hubbard R, Fleming KM, Smith CJ, West J. Bullous pemphigoid and pemphigus vulgaris–incidence and mortality in the UK: population based cohort study. BMJ 2008;337:a180.
4. Gudi VS, White MI, Cruickshank N, Herriot R, Edwards SL, Nimmo F, et al. Annual incidence and mortality of bullous pemphigoid in the Grampian Region of North-east Scotland. Br J Dermatol 2005;153:424-427.
5. Stanley JR, Hawley-Nelson P, Yuspa SH, Shevach EM, Katz SI. Characterization of bullous pemphigoid antigen: a unique basement membrane protein of stratified squamous epithelia. Cell 1981;24:897-903.
6. Sami N, Bhol KC, Beutner EH, Plunkett RW, Leiferman KM, Foster CS, et al. Simultaneous presence of mucous membrane pemphigoid and pemphigus vulgaris: molecular characterization of both autoantibodies. Clin Immunol 2001;100:219-227.
7. Sami N, Ahmed AR. Dual diagnosis of Pemphigus and pemphigoid. Retrospective review of thirty cases in the literature. Dermatology 2001;202:293-301.
8. Schmidt E, Zillikens D. Modern diagnosis of autoimmune blistering skin diseases. Autoimmun Rev 2010;10:84-89.
9. Labib RS, Anhalt GJ, Patel HP, Mutasim DF, Diaz LA. Molecular heterogeneity of the bullous pemphigoid antigens as detected by immunoblotting. J Immunol 1986;136:1231-1235.
10. Kasperkiewicz M, Zillikens D. The pathophysiology of bullous pemphigoid. Clin Rev Allergy Immunol 2007;33:67-77.
11. Büdinger L, Borradori L, Yee C, Eming R, Ferencik S, Grosse-Wilde H, et al. Identification and characterization of autoreactive T cell responses to bullous pemphigoid antigen 2 in patients and healthy controls. J Clin Invest 1998;102:2082-2089.
12. Romagnani S. Human TH1 and TH2 subsets: regulation of differentiation and role in protection and immunopathology. Int Arch Allergy Immunol 1992;98:279-285.
13. Delgado JC, Turbay D, Yunis EJ, Yunis JJ, Morton ED, Bhol K, et al. A common major histocompatibility complex class II allele HLA-DQB1* 0301 is present in clinical variants of pemphigoid. Proc Natl Acad Sci U S A 1996;93:8569-8571.
14. Esmaili N, Mortazavi H, Chams-Davatchi C, Daneshpazhooh M, Damavandi MR, Aryanian Z, et al. Association between HLA-DQB1*03:01 and Bullous pemphigoid in Iranian patients. Iran J Immunol 2013;10:1-9.
15. Okazaki A, Miyagawa S, Yamashina Y, Kitamura W, Shirai T. Polymorphisms of HLA-DR and -DQ genes in Japanese patients with bullous pemphigoid. J Dermatol 2000;27:149-156.
16. Gao XH, Winsey S, Li G, Barnardo M, Zhu XJ, Chen HD, et al. HLA-DR and DQ polymorphisms in bullous pemphigoid from northern China. Clin Exp Dermatol 2002;27:319-321.
17. Lin MS, Fu CL, Giudice GJ, Olague-Marchan M, Lazaro AM, Stastny P, et al. Epitopes targeted by bullous pemphigoid T lymphocytes and autoantibodies map to the same sites on the bullous pemphigoid 180 ectodomain. J Invest Dermatol 2000;115:955-961.
18. Dabelsteen E, Ullman S, Thomsen K, Rygaard J. Demonstration of basement membrane autoantibodies in patients with benign mucous membrane pemphigoid. Acta Derm Venereol 1974;54:189-192.
19. Weber R, Monteiro F, Preuhs-Filho G, Rodrigues H, Kalil J, Miziara ID. HLA-DRB1*04:02, DRB1*08:04 and DRB1*14 alleles associated to pemphigus vulgaris in southeastern Brazilian population. Tissue Antigens 2011;78:92-93.