

Tunisian endemic pemphigus foliaceus is associated with the HLA-DR3 gene: anti-desmoglein 1 antibody-positive healthy subjects bear protective alleles

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Summary

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Background Pemphigus foliaceus is an autoimmune blistering skin disease that partly results from genetic factors, especially human leucocyte antigen (HLA) class II genes.

Objectives The aim of the study was to determine the HLA DR/DQ markers of susceptibility and protection in the Tunisian endemic form.

Methods Genomic DNA from 90 patients with pemphigus foliaceus recruited from all parts of the country and matched by age, sex and geographical origin with 270 healthy individuals, was genotyped.

Results Firstly, when the whole patient population was studied, DRB1*03, DQB1*0302 and DRB1*04 alleles were significantly associated with the disease while a significant decrease of, in particular, DRB1*11 and DQB1*0301 was observed in patients compared with controls. DRB1*0301 was the dominant allele in DR3-positive patients and controls, while DRB1*0402 was found in 42% of DR4-positive patients. Secondly, when the HLA DR/DQ allele distribution was studied after dividing patients according to their geographical origin, the southern group, which consisted exclusively of patients with the endemic form of the disease, showed the same associations as the whole pemphigus foliaceus population, particularly with DRB1*03. In the northern group, only the DRB1*04 and DQB1*0301 alleles were found to be associated. Interestingly, anti-desmoglein 1 antibody-positive healthy controls did not carry susceptibility alleles but, in contrast, most carried negatively associated alleles. **Conclusions** These observations indicate that a particular genetic background characterizes the Tunisian endemic form of pemphigus foliaceus and that HLA class II genes control the pathogenic properties of the autoimmune response rather than the initial breakage of B-cell tolerance.

Pemphigus foliaceus (PF) is an autoimmune disease characterized by blisters due to acantholysis of the most superficial epidermal cells. The autoimmune process results in the production of autoantibodies, mainly of the IgG₄ subclass, directed against a desmosomal glycoprotein known as desmoglein (Dsg) 1 and responsible for the acantholytic process.¹ PF is considered to be a multifactorial disease resulting from the interaction of environmental factors with multiple genes. It occurs in different areas of the world in a sporadic or endemic form. In 1993, Morini *et al.*² reported for

the first time the endemic Tunisian form, observed in the south of the country. Several features characterize this form: a significantly higher incidence rate compared with northern countries, occurrence mainly in young adult women and the absence of cases during childhood.^{3,4} Recently, we showed that anti-Dsg1 antibodies could be detected in healthy controls living in Tunisia and that the prevalence of anti-Dsg1 antibody-positive subjects was higher in the southern regions (9.23% vs. 5.71% in the north) and could reach 22% in the most southern rural areas (personal data). Finally, the

discovery of cases among genetically related household members, especially in southern Tunisia,⁵ strongly suggests the involvement of genetic factors in this form of PF.

In pemphigus, the search for susceptibility genes has used the candidate gene strategy through case-control association studies, and involved genes implicated either in pemphigus physiopathology⁶ or in the autoimmune response.⁷⁻⁹ In this regard, associations between human leucocyte antigens (HLA) and PF have long been recognized in different ethnic populations. However, no data exist concerning the Tunisian endemic form of PF. Thus, the aim of our study was to determine the HLA class II markers of susceptibility and protection in this type of pemphigus.

Materials and methods

Patients and controls

In this case-control study we enrolled 90 patients with PF, 50 from the south and 40 from the north attending the Dermatology Department of the University Hospitals of Tunis in the north and Sfax in the south. The demographic features of our patient groups differed between the north and the south: the disease was associated in the south with an important sex ratio disequilibrium (female/male, 9/1 in the south vs. 2.33/1 in the north) and a lower mean age of disease onset (34 years in the south vs. 45 years in the north). The period of recruitment started in 1998 in northern regions and in 2002 in southern areas. All patients with PF were diagnosed on clinical, histological and immunological criteria¹⁰ and were matched by age (± 5 years), sex and geographical origin to 270 healthy controls (130 from the south and 140 from the north). All patients and healthy controls gave written consent to participate in the study.

Genotyping methods

Genomic DNA, from peripheral blood lymphocytes collected on ethylenediamine tetra-acetic acid, was extracted using Qiagen kits (Qiagen Inc., Valencia, CA, U.S.A.). The generic DR-DQ typing and the DR3/DR4 subtyping were performed by a polymerase chain reaction-sequence specific primer (PCR-SSP) technique using One Lambda kits (One Lambda Inc., Canoga Park, CA, U.S.A.). Polymorphic fragments of HLA-DRB1, DRB3, DRB4, DRB5 and DQB1 were amplified by a PCR reaction. The obtained fragments were detected in a 1.5% agarose gel, stained with ethidium bromide and visualized under ultraviolet illumination. The reaction results were read and analysed using the kit instructions.

Anti-desmoglein 1 antibody measurement

Anti-Dsg 1 antibodies were detected by a commercial enzyme-linked immunosorbent assay (ELISA) kit (MBL, Nagoya, Japan) according to the manufacturer's instructions. The MESACUP Dsg1 test is a semiquantitative ELISA used for the detection of IgG anti-Dsg1 antibodies in human sera. The

antibody titre was expressed as an index value (U mL^{-1}); the cut-off value was set at 14 U mL^{-1} .

Statistical analysis

The frequencies of HLA class II gene polymorphic alleles in patients and controls were compared using the chi-square test. $P \leq 0.05$ was considered statistically significant. Significant P -values were corrected (P_c) by the number of alleles tested at each locus (13 for DRB1* and seven for DQB1*) (Bonferroni's corrections). Relative risks were calculated as odds ratios (OR) using 2×2 contingency tables. The linkage disequilibrium parameters for these antigens (δ) were calculated using 2×2 tables.

Results

DRB1 and DQB1 generic and allelic frequencies in Tunisian patients and controls

Analysis of DRB1 polymorphism by low-resolution polymerase chain reaction-sequence-specific primer typing

A highly significant increase of DRB1*04 allele frequency ($P_c = 6 \times 10^{-6}$, OR 2.7) and, at a lower level, of DRB1*03 ($P_c = 0.01$, OR 2) was observed in patients, reaching, respectively, 31.7% and 25.6% compared with 14.6% and 14.6% in the controls (Table 1).

The study of other DRB1 alleles showed a significant decrease of DRB1*11 ($P_c = 3 \times 10^{-4}$, OR 0.15) and DRB1*13 ($P_c = 0.04$, OR 0.37) allele frequencies, reaching, respectively, 2.2% and 5.6% in patients compared with the values of 13.5% and 13.7% in the control population (Table 1).

Analysis of DRB1*03- and DRB1*04-positive subjects by high-resolution polymerase chain reaction-sequence-specific primer typing

All subjects carrying DR3 and/or DR4 were subtyped. Except for DRB1*0404, which was found only in patients and was significantly associated with Tunisian PF ($P = 0.007$), and DRB1*0405, which was significantly ($P = 0.02$) more frequent in controls (23.9%) compared with patients (8.7%), no significant differences were found in the subtype distribution between patients with PF and controls. DRB1*0301 was the dominant allele in DR3-positive patients (48.8%) and healthy controls (45%) (Table 2).

Among DR4-positive subjects, DRB1*0402 was the most frequent subtype in patients (23.1%), while DRB1*0405 was the most frequent subtype in controls (23.9%) (Table 2).

Analysis of DQB1 polymorphism

We noted a significant increase of DQB1*0302 allele frequency ($P_c = 2 \times 10^{-3}$), which reached 25.6% in patients with PF vs. 14.4% in controls, whereas, a significant decrease of DQB1*0301 ($P_c = 3 \times 10^{-5}$) and DQB1*06 ($P_c = 0.02$) was found in patients with PF.

Table 1 Distribution of significant DR/DQ alleles in Tunisian patients with pemphigus foliaceus and controls

Generic DRB1/DQB1 ^a	Whole population				Southern group				Northern group			
	Allele frequencies (%)				Allele frequencies (%)				Allele frequencies (%)			
	Patients (n = 90)	Controls (n = 270)	OR	P _c	Patients (n = 50)	Controls (n = 130)	OR	P _c	Patients (n = 40)	Controls (n = 140)	OR	P _c
DRB1*03	25.6	14.63	2	0.01 ^b	29	11.15	3.25	0.00048 ^b	21.3	17.86	1.24	1
DRB1*04	31.7	14.63	2.7	0.000006 ^c	32	13.48	3	0.0007 ^b	31.4	15.71	2.44	0.02 ^b
DQB1*11	2.2	13.52	0.15	0.0003 ^b	1	12.31	0.07	0.01 ^c	3.8	14.64	0.23	0.11
DRB1*13	5.6	13.70	0.37	0.04 ^c	7	18.46	0.33	0.09	3.8	9.29	0.38	1
DRB1*15	4.4	10.37	0.40	0.20	1	10.38	0.09	0.04 ^c	8.8	10.36	0.83	1
DQB1*0301	3.9	17.22	0.19	0.00003 ^c	4	16.54	0.21	0.007 ^b	3.8	18.21	0.17	0.007 ^c
DQB1*0302	25.6	14.44	2.03	0.002 ^b	25	10.77	2.76	0.004 ^c	26.3	17.5	1.68	0.41
DQB1*06	10	19.63	0.45	0.02 ^c	8	22.69	0.3	0.013 ^c	12.5	16.79	0.71	1

^aWe also tested the DRB1*01, DRB1*07, DRB1*08, DRB1*09, DRB1*10, DRB1*12, DRB1*14, DRB1*16, DQB1*02, DQB1*0303, DQB1*04 and DQB1*05 alleles which were not found to be associated; ^bsusceptibility allele; ^cprotective allele. P_c, probability corrected with Bonferroni's correction; OR, odds ratio.

Table 2 DRB1 subtype frequencies in DR3- and DR4-positive patients with pemphigus foliaceus and controls

HLA-DR3/DR4 alleles	Whole population			Southern group			Northern group		
	Patients n (%)	Controls n (%)	P-value	Patients n (%)	Controls n (%)	P-Value	Patients n (%)	Controls n (%)	P-value
DRB1*03	46	79		29	29		17	50	
DRB1*0301	41 (48.8)	63 (45)	NS	25 (50)	22 (42.31)	NS	16 (47.1)	41 (46.59)	NS
DRB1*0302	1 (1.2)	9 (6.43)	NS	1 (2)	5 (9.62)	NS	0 (0)	4 (4.55)	NS
DRB1*0305	1 (1.2)	1 (0.71)	NS	0 (0)	0 (0)	NS	0 (0)	1 (1.14)	NS
DRB1*0306	0 (0)	1 (0.71)	NS	1 (2)	1 (1.92)	NS	0 (0)	0 (0)	NS
DRB1*0307	1 (1.2)	3 (2.14)	NS	0 (0)	0 (0)	NS	0 (0)	1 (1.14)	NS
DRB1*0308	0 (0)	1 (0.71)	NS	1 (2)	1 (1.92)	NS	0 (0)	2 (2.27)	NS
DRB1*0312	0 (0)	1 (0.71)	NS	0 (0)	0 (0)	NS	0 (0)	1 (1.14)	NS
DRB1*0315	1 (1.2)	0 (0)	NS	0 (0)	0 (0)	NS	1 (2.9)	0 (0)	NS
DRB1*0328	1 (1.2)	0 (0)	NS	1 (2)	0 (0)	NS	0 (0)	0 (0)	NS
DRB1*04	57	79		32	35		25	44	
DRB1*0401	0 (0)	3 (2.24)	NS	0 (0)	0 (0)	NS	0 (0)	3 (3.95)	NS
DRB1*0402	24 (23.1)	20 (14.93)	NS	10 (16.7)	10 (17.42)	NS	14 (31.8)	10 (13.16)	0.01 ^a
DRB1*0403	4 (3.8)	8 (5.97)	NS	2 (3.3)	6 (10.34)	NS	2 (4.5)	2 (2.63)	NS
DRB1*0404	7 (6.7)	0 (0)	0.007 ^a	3 (5)	0 (0)	NS	4 (9.1)	0 (0)	0.03 ^a
DRB1*0405	9 (8.7)	32 (23.88)	0.002 ^b	7 (11.7)	6 (10.34)	NS	2 (4.5)	26 (34.21)	0.0002 ^b
DRB1*0406	8 (7.7)	4 (2.99)	NS	7 (11.7)	3 (5.17)	NS	1 (2.3)	1 (1.32)	NS
DRB1*0407	0 (0)	2 (1.49)	NS	0 (0)	0 (0)	NS	0 (0)	2 (2.63)	NS
DRB1*0408	2 (1.9)	0 (0)	NS	1 (1.7)	0 (0)	NS	1 (2.3)	0 (0)	NS
DRB1*0413	0 (0)	1 (0.75)	NS	0 (0)	1 (1.72)	NS	0 (0)	0 (0)	NS
DRB1*0414	0 (0)	1 (0.75)	NS	0 (0)	1 (1.72)	NS	0 (0)	0 (0)	NS
DRB1*0427	2 (1.9)	0 (0)	NS	1 (1.7)	0 (0)	NS	1 (2.3)	0 (0)	NS
DRB1*0441	0 (0)	1 (0.75)	NS	0 (0)	1 (1.72)	NS	0 (0)	0 (0)	NS
DRB1*0446	0 (0)	6 (4.48)	NS	0 (0)	6 (10.34)	NS	0 (0)	0 (0)	NS
DRB1*0448	0 (0)	1 (0.75)	NS	0 (0)	1 (1.72)	NS	0 (0)	0 (0)	NS
DRB1*0452	1 (1)	0 (0)	NS	1 (1.7)	0 (0)	NS	0 (0)	0 (0)	NS

^aSusceptibility allele; ^bprotective allele.

NS, not significant.

In summary, the DRB1*03, DRB1*04 and DQB1*0302 alleles seem to be contributing to disease susceptibility whereas the DRB1*11, DRB1*13 and DQB1*0301 alleles seem to be protective.

Haplotype and linkage disequilibrium

Because of the strong linkage disequilibrium between the DR and DQ loci, 24 (23%) patients with PF with the DRB1*0301

allele also carried the DQB1*02 allele and 11 (10.6%) patients with the DRB1*0402 allele carried the DQB1*0302 allele, suggesting that the presumed haplotypes of susceptibility to Tunisian PF are DRB1*0301-DQB1*02 ($P = 0.001$, OR 2) and DRB1*0402-DQB1*0302 ($P = 0.0001$, OR 2.75).

In contrast, the DRB1*11-DQB1*0301 haplotype seems to confer protection against PF ($P = 0.0005$, OR 0.12).

DRB1 and DQB1 allele frequencies in southern and northern Tunisian patients with pemphigus foliaceus

As previous studies have shown that PF displays different epidemiological characteristics in the south and the north of Tunisia,^{2,4} we next determined the frequency of HLA-class II alleles in southern and northern patients with PF and their respective matched controls separately in order to look for a possible difference in their genetic background. Table 1 shows that the distribution of DRB1- and DQB1-associated alleles in these two groups was very different.

Analysis of DRB1 polymorphism by low-resolution polymerase chain reaction–sequence-specific primer typing

The southern group conserved all generic alleles found to be positively associated with the whole PF population. Notably, DRB1*03 was confirmed as a susceptibility allele only in the southern group, and interestingly, with an even higher significance ($P_c = 0.00048$, OR 3.25) than in the whole population ($P_c = 0.01$, OR 2). Furthermore, DRB1*15 ($P_c = 0.004$, OR 0.09) was shown to be a new negatively associated allele.

In the northern group, and among these alleles, only DRB1*04 as a susceptibility allele and DQB1*0301 as a negatively associated allele were found.

Analysis of DRB1*03- and DRB1*04-positive subjects by high-resolution polymerase chain reaction–sequence-specific primer typing

HLA-DRB1*0402 and DRB1*0404 subtypes of DRB1*04 were found to be significantly increased among DRB1*04-positive northern patients with PF compared with controls ($P = 0.013$, OR 3 and $P = 0.03$, respectively). Whereas, DRB1*0405 was significantly more frequent in northern controls ($P = 0.0002$, OR 0.09) (Table 2).

In contrast, no significant differences were found in the subtype distribution between southern patients and their control group (Table 2).

Analysis of DQB1 polymorphism

DQB1*0302 as a susceptibility allele ($P_c = 4 \times 10^{-3}$, OR 2.76) and DQB1*06 as a negatively associated allele ($P_c = 0.013$, OR 0.3) were found only in southern patients.

A significant decrease of DQB1*0301 allele frequency was observed in both groups.

HLA class II alleles in anti-desmoglein 1 antibody-positive healthy controls

The demonstration that HLA class II alleles are associated with Tunisian PF has various implications: the alleles may contribute to the initial breakage of B-cell tolerance to Dsg1 or to the triggering of the clinical phase of the disease. To determine if HLA class II alleles confer susceptibility to Tunisian PF and/or participate in anti-Dsg1-antibody production, we investigated whether or not anti-Dsg1 antibody-positive healthy controls express particular HLA class II alleles. We observed that among the 270 healthy controls, 20 (7.4%) had abnormal anti-Dsg1-antibody titres; of these 12 and eight came from the southern and northern populations, respectively. Index values ranged from 15.4 to 169 U mL⁻¹ (mean $\pm$ SD, 32 ± 47) and from 18 to 119 U mL⁻¹ (43.6 ± 37) for southern and northern controls, respectively (Table 3).

The analysis of HLA molecular typing in anti-Dsg1 antibody-positive and -negative healthy controls did not show any significant association between the expression of Tunisian PF HLA class II susceptibility alleles and the production of anti-Dsg1 autoantibodies. However, when we examined the HLA class II subtype distribution allele by allele, only one healthy control with anti-Dsg1 antibodies had susceptibility alleles without any protective alleles, while the majority (18/20) expressed negatively associated alleles (Table 3).

Table 3 Anti-desmoglein 1 (anti-Dsg1)-antibody titres and HLA class II haplotypes of healthy controls with anti-Dsg1 antibodies

Healthy control	Anti-Dsg1 titre (U mL ⁻¹)	HLA-DR/DQ haplotypes ^a
1	19	DRB1*08/*15-DQB1*0301/*06
2	20	DRB1*0302/*02-DQB1*02/*0301
3	16	DRB1*13/*13-DQB1*0301/*02
4	169	DRB1*07/*07-DQB1*02/*02
5	31	DRB1*13/*14-DQB1*05/*06
6	81	DRB1*13/*15-DQB1*06/*06
7	21	DRB1*11/*13-DQB1*05/*06
8	20	DRB1*0301/*13-DQB1*02/*05
9	16	DRB1*11/*11-DQB1*05/*05
10	19	DRB1*0403/*15-DQB1*0302/*06
11	19	DRB1*13/*0405-DQB1*04/*0301
12	30	DRB1*11/*11-DQB1*0302/*0302
13	119	DRB1*0302/*14-DQB1*0301/*04
14	33	DRB1*0301/*15-DQB1*02/*06
15	24	DRB1*0405/*13-DQB1*06/*06
17	19	DRB1*01/*0407 DQB1*0302/*05
18	119	DRB1*11/*0405 DQB1*0302/*0302
18	18	DRB1*11/*11 DQB1*0301/*05
19	20	DRB1*11/*11 DQB1*0301/*0301
20	59	DRB1*11/*0301 DQB1*02/*0301

^aHLA DR/DQ negatively associated alleles are underlined; HLA DR/DQ susceptibility alleles are in bold.

Pocket specificity of pemphigus foliaceus-associated DRB1 molecules

Polymorphic amino acid residues, present in five different pockets of DR β chains (P1, P4, P6, P7, P9), control the specificity of the peptide-binding site of the DR molecules and thus the array of antigenic peptides presented to T cells. Common structural features have been described for the pockets of DR molecules previously shown to be associated with endemic and sporadic PF. Table 4 shows that the DR β chains of the two susceptibility alleles DRB1*0402 and *0404 have the same hydrophobic residue (V) at P1, and negatively charged residues (D or E) at positions DR β 28 of the P7 pocket and DR β 9 and 57 of the P9 pocket. Interestingly, the DR β chain of the dominant allele of DR3-positive patients, DRB1*0301, shares the same residues at these positions. In particular, residue 57 which is known to be associated with autoimmune diseases is an aspartic acid in all PF-associated molecules. In the DR β chain of the negatively associated allele DRB1*0405, this residue is a serine, while the valine of the P1 pocket is replaced by a glycine.

Discussion

It has been well established that the HLA class II locus participates in PF susceptibility. Several alleles have been demonstrated to be positively associated with PF, among which DRB1*04 has been consistently reported to be a susceptibility allele for both the sporadic and the Brazilian endemic form of PF. Also, DQB1*0302 seems to be an allele common to all forms of pemphigus observed in the world.^{11,12} In fogo selvagem, susceptibility appears to be mostly related to DRB1*0102, *0404, *0406, *1406, *1601 and DQB1*0302.^{11,13} In the sporadic form, studies performed in France also showed an association with DRB1*0102 and DRB1*0404^{12,14} while an association with pemphigus vulgaris susceptibility alleles was observed in Japanese (DRB1*0406 and DRB1*1401) and in Italian (DRB1*0404 and DRB1*1401)¹⁴ patients with PF. These data suggest that common genetic factors contribute to the different varieties of PF, which share similar immunological characteristics but occur in different environments. Tunisian

endemic PF constitutes a distinct variety of the disease with particular and unique epidemiological characteristics as the disease occurs mainly in young women at a mean age of 35 years and spares children. It is thus questionable if the genetic factors previously shown to be associated with the other varieties of PF also contribute to the occurrence of the disease in Tunisia.

A first striking observation was made. Besides the HLA class II alleles previously found to confer susceptibility to PF (DR4 and DQB1*0302), this study indicates that DR3 constitutes an allele of susceptibility in Tunisian PF. This is surprising as, so far, DRB1*03 has never been reported to be associated with the endemic or the sporadic form of PF, but in contrast the DRB1*0301 subtype was recently shown to be negatively associated with the Brazilian form of PF.¹³ In our study, this association was observed with a weak significance ($P_c = 0.01$) in the whole selected PF population originating from both the north and the south of Tunisia. Thus to confirm the association of DRB1*03 with Tunisian PF and to eliminate confounding factors which may provide spurious results, we analysed the distribution of HLA class II alleles in a more homogeneous PF population, namely in patients originating from the north (where the sporadic form occurs) or from the south (where endemic PF occurs) separately. This analysis revealed that DRB1*03 is not associated with PF in the north, while, it is associated with a very high significance in the southern group ($P_c = 0.00048$). Thus, the DRB1*03 allele can be considered to be the main and characteristic susceptibility allele of the endemic Tunisian form of PF. In this regard, it is surprising that even though DRB1*04 was confirmed as a susceptibility allele with a higher significance in the south ($P_c = 0.007$) than in the north ($P_c = 0.02$), only in the northern group was DRB1*0404 confirmed to be a susceptibility allele and DRB1*0405 was confirmed as a negatively associated allele. While DQB1*0302 was confirmed only in the south, the northern group showed a new susceptibility allele, DRB1*0402. In addition to DRB1*11 and DQB1*06, the southern group revealed a new negatively associated allele, DRB1*15.

These differences in the susceptibility/protection alleles between the two groups provide an additional argument

Table 4 Polymorphic residues of DR β 1 chain forming the pockets of the DR molecules associated with Tunisian pemphigus foliaceus

	AA position															
	P1			P4			P6			P7			P9			
DRB1*	86	13	70	71	74	78	11	28	30	47	67	71	9	37	57	60
03 ^a	V	S	Q	<u>K</u>	<u>R</u>	Y	S	D	Y	F	L	<u>K</u>	E	N	D	Y
0402	V	H	D	E	A	Y	V	D	Y	Y	I	E	E	Y	D	Y
0404	V	H	Q	<u>R</u>	A	Y	V	D	Y	Y	L	<u>R</u>	E	Y	D	Y
0405	G	H	Q	<u>R</u>	A	Y	V	D	Y	Y	L	<u>R</u>	E	Y	S	Y

The amino acid (AA) residues are designated according to the letter code: D, E negatively charged AA, are in bold. K, R positively charged AA, are underlined. ^aThe sequence shown is that of the DRB1*0301 molecule found in almost 90% of DR3-positive patients.

supporting the view that PF occurs in two forms in Tunisia, a sporadic form in northern regions and an endemic form in southern areas, the endemic form exhibiting characteristic epidemiological features and a particular genetic background.

The association of DRB1*03 with autoimmune bullous skin diseases has previously been reported, as it was shown to constitute a susceptibility allele in paraneoplastic pemphigus (PNP).¹⁵ In this rare form of pemphigus, characterized by an association with lymphoproliferative disorders and unique clinical features, autoantibodies not only target proteins of the plakins family but also Dsg1 and Dsg3. Thus, the fact that PF and PNP share the same allele of susceptibility suggests that the breakage of B-cell tolerance in PNP may initially involve Dsg1 and subsequently extend to other desmosomal components.

It is well established that the structure of DR β 1 chains accounts for their ligand specificity. In particular, five pockets P1, P4, P6, P7 and P9 participate in their peptide-binding properties.¹⁶ More precisely, the pocket specificity is determined by the presence of a list of amino acids required or preferred at a particular anchor. We thus expected the amino acids of the peptide-binding pockets of the DR β chains repeatedly shown to be associated with PF to be similar if not identical to those of the DRB1*03 β chain. Indeed, this was the case: this β chain shares similar amino acid binding characteristics at crucial anchor positions with β chains of the DR4 susceptibility alleles, suggesting that the DRB1*03 molecule can accommodate and present Dsg1 peptide(s) in the same manner as other susceptibility alleles.

The analysis of HLA molecular typing in anti-Dsg1 antibody-positive healthy controls did not find any significant association between the expression of Tunisian PF HLA class II susceptibility alleles and the production of anti-Dsg1 autoantibodies. In contrast, the majority of anti-Dsg1-positive healthy controls (18/20) bore at least one negatively associated allele. The latter observation constitutes a strong argument supporting the view that DR alleles shown to be negatively associated with PF could confer protection.

Taken together, these findings allow us to assign to HLA class II alleles a new role in autoimmune bullous skin disorders: HLA DR/DQ molecules are not involved in the initial breakage of tolerance to Dsg1 but control the transition from an asymptomatic and nonpathogenic autoantibody response to the clinical phase of the disease and the production of anti-Dsg1 antibodies with constant and variable region-dependent pathogenic properties.

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