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ORIGINAL ARTICLE

Anti-Desmoglein 1 and 3 Autoantibody Levels in Endemic Pemphigus Foliaceus and Pemphigus Vulgaris from Brazil

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SUMMARY

Background: **Pemphigus is a group of autoimmune blistering diseases of which the major forms are pemphigus foliaceus (PF) and vulgaris (PV). In Brazil, PF occurs in an endemic form also known as *fogo selvagem*. The main autoantibody in PF is against desmoglein 1 (DSG1), while in PV the main antibody is anti-desmoglein 3 (DSG3), but often anti-DSG1 is also present. The aim of the present study was to analyze the levels of anti-DSG1 and anti-DSG3 autoantibodies in Brazilian PF and PV patients, considering different stages of the disease for PF patients and comparing these levels to those of healthy individuals living in and outside the endemic regions.**

Methods: **Levels of anti-DSG1 and anti-DSG3 were measured in the sera of Brazilian PF (n = 68) and PV (n = 20) patients as well as in clinically healthy (control) individuals (n = 48) by Enzyme Linked Immunosorbent Assay (ELISA). Comparisons were made using Kruskal-Wallis and Mann-Whitney tests.**

Results: **As expected, anti-DSG1 was more prevalent among PF patients (84% against 43% in PV), while anti-DSG3 was more prevalent in PV patients (50% against 4% in PF). Levels of anti-DSG1 in PF patients in remission differed from those in patients undergoing active disease (p = 0.003), and patients in long-term remission (more than two years without presenting new lesions) were similar to control individuals living in the endemic region and surrounding area (p = 0.09). Moreover, patients with a more severe form of the disease had higher levels of anti-DSG1 (at least 134 U/mL, mean of 233 U/mL) than patients with a less severe form (fewer lesions) (mean of 193 U/mL, including two negative individuals).**

Conclusions: **Despite the importance of these antibodies for diagnosis and management purposes, their presence in a healthy individual and in patients under remission indicates that caution should be taken when using anti-DSG for diagnosis, especially in endemic areas.**

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INTRODUCTION

Pemphigus foliaceus (PF) and pemphigus vulgaris (PV) are the major types of pemphigus, a group of autoimmune diseases of the epidermis characterized by acantholysis, the rupture of the cellular junctions among ke-

ratinocytes, resulting in epidermal blisters and erosions. In PF, skin lesions are superficial, while in PV the blisters originate in deep layers of the epidermis. Pemphigus patients develop autoantibodies against various self-antigens [1-4]. Of these, pathogenic antibodies against the ectodomains of desmogleins (DSG) are the most important. The desmogleins are calcium-dependent cell-adhesion glycoproteins of the desmosome and members of the major cadherin gene family. In PF, the main autoantibody is against DSG1 [5]. PV patients with only mucosal lesions tend to have autoantibodies against DSG3, whilst PV patients with mucocutaneous lesions usually have autoantibodies against both DSG1 and DSG3 [6].

Pemphigus occurs at low incidence worldwide, and PV is the major form in most geographic regions. Yet, PF is endemic in Brazil, especially in the midwestern part of the country [7]. Brazilian endemic PF is also known as *fogo selvagem*, and this is the form of PF analysed in the present study. In the endemic region, also individuals without pemphigus may have antibodies against desmogleins [8,9], indicating that an environmental factor triggers the immune response against desmogleins, but the pathogenic response only evolves in genetically predisposed individuals. Interestingly, a rise of incidence of PV in some regions where PF is endemic has been reported, leading to the suggestion of an endemic form of PV [10,11].

In the present study, we aimed to verify the levels of anti-DSG1 and anti-DSG3 in PF and PV patients and in healthy individuals that reside both in and outside of the endemic region.

MATERIALS AND METHODS

The patient sample was composed of 68 PF patients and 20 PV patients. Controls (n = 48) were individuals with no autoimmune disease and no consanguineous relatives with pemphigus. For nine patients (8 PF and 1 PV), more than one serum sample was available. Blood samples were collected between 2011 and 2014 in one of the following locations: Hospital Adventista do Pênfigo (HAP), Campo Grande, state of Mato Grosso do Sul; Hospital das Clínicas of the Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (FMRP), Ribeirão Preto, state of São Paulo; Lar da Caridade Hospital do Fogo Selvagem de Uberaba (HFSU), Uberaba, state of Minas Gerais; Hospital de Clínicas and Laboratório de Genética Molecular Humana of the Universidade Federal do Paraná (UFPR), Curitiba, state of Paraná (Supplementary Table 1). After serum isolation, samples were stored at -70ºC until use. Healthy control individuals were subdivided into individuals from the endemic region and surroundings (CE, n = 9) and individuals living outside the endemic region (Controls, n = 39).

Enzyme Linked Immunosorbent Assay (ELISA) kits, MBL® (RG-M7593-D and RG-7680-EC-D) were used to measure anti-DSG1 (in all samples) and anti-DSG3 (in 50 patients and 33 control individuals) levels in the serum, following manufacturer's instructions. The cutoff value for a sample to be considered positive is 20 U/mL. Some samples were tested at least twice (22 for anti-DSG1, and 2 for anti-DSG3), providing very similar results (maximum standard deviation of 1.9 U/mL among negative individuals, and of 49 U/mL among positives, never changing the interpretation). For analysis, PF patients were divided into four subgroups: patients presenting lesions and under immunosuppressive treatment on blood sampling (PFCLCT, n = 43), patients presenting lesions in the absence of immunosuppressive treatment (PFCLST, n = 5); patients without lesions and under immunosuppressive treatment (PFSLCT, n = 11); and patients without lesions and not under immunosuppressive treatment (PFSLST, n = 14). All PV patients had lesions and were under immunosuppressive treatment when contacted. In addition, PF patients were classified according to severity of lesions or time without presenting new lesions: 1) patients with many skin lesions (n = 6); 2) patients with few isolated lesions, or cicatrizing lesions (n = 42); 3) patients not presenting new lesions for a maximum of 18 months (n = 14); 4) patients not presenting new lesions for more than two years (n = 7). PV patients were classified in mucosal only (n = 10), cutaneous only (n = 4), and mucocutaneous (n = 4), according to the location of the lesions at the moment of blood sampling.

When different samples were available for the same individual, if conditions were similar between the occasions (i.e., individual with the same age, patient under similar immunosuppressive dosage and similar severity of the disease), a mean of the data was used in the analysis. However, if conditions differed, the different data were kept for analysis (Supplementary Table 1). Spearman's correlation was estimated between age and antibody levels [12]. As the correlation was low (rho = -0.29, p = 0.0145), the age was not considered in further analysis.

Autoantibody levels were compared among subgroups by Kruskal-Wallis and Mann-Whitney tests, in R platform, package *stats* versions 2.15.3 and 3.2.0 [12]. Kruskal-Wallis was also used to compare antibody levels among patients under different doses of immunosuppressive treatment (Prednisone). Box plot and dot plot graphs were generated in R, packages *fields* version 3.3.1 and *graphics* version 3.2.0 [12].

RESULTS

Anti-DSG1 and anti-DSG3 levels were measured in PF and PV patients as well as in control individuals (Supplementary Table 1). Of the clinically healthy (control) individuals, only one living in an endemic region was positive for both autoantibodies (49 U/mL of anti-DSG1 and 64 U/mL of anti-DSG3). The other control individuals were negative for anti-DSG1 and, when tested, also

Table 1. P-values for pairwise comparisons of anti-DSG1 levels in serum of subgroups of pemphigus patients and control individuals.

	CE (n = 9)	Controls (n = 39)	PFCLCT (n = 43)	PFCLST (n = 5)	PFSLCT (n = 11)	PFSLST (n = 14)
Controls	0.2193					
PFCLCT	0.00001	1.60E-13				
PFCLST	0.0033	0.0003	0.6855			
PFSLCT	0.0014	0.00001	0.5055	0.4278		
PFSLST	0.0546	0.0002	0.000005	0.0035	0.0041	
PV (n = 21)	0.0145	0.000001	3.40E-08	0.0011	0.0020	0.9731

CE - clinically healthy individuals that live in the endemic region, Controls - clinically healthy individuals living outside the endemic region, PFCLCT - patients presenting lesions and under immunosuppressive treatment, PFCLST - patients presenting lesions but not under immunosuppressive treatment, PFSLCT - patients not presenting lesions and under immunosuppressive treatment, PFSLST - patients not presenting lesions and not under immunosuppressive treatment, n - number of individuals.

Table 2. P-values for pairwise comparisons of anti-DSG3 levels in serum of pemphigus patients and control individuals.

	CE (n = 8)	Controls (n = 25)	PF (n = 46)
Controls	0.0372		
PF	0.7886	0.0045	
PV (n = 8)	0.0406	0.0006	0.0035

CE - clinically healthy individuals that live in the endemic region, Controls - clinically healthy individuals living outside the endemic region, PF - pemphigus foliaceus patients, PV - pemphigus vulgaris patients, n - number of individuals.

Table 3. P-values for pairwise comparisons of anti-DSG1 levels in PF patients classified according to severity of lesions or time without lesions and control individuals from the endemic region.

	1 (n = 6)	2 (n = 42)	3 (n = 14)	CE (n = 9)
2	0.11542			
3	0.01188	0.048		0.0089
4 (n = 7)	0.00341	0.0002	0.07957	0.0901

1) patients with many skin lesions, 2) patients with few isolated lesions, or cicatrizing lesions, 3) patients not presenting new lesions for a maximum of 18 months, 4) patients not presenting new lesions for more than 2 years, CE - control individuals that live in the endemic region, n – number of individuals.

negative for anti-DSG3. Anti-DSG1 antibody was observed in most PF patients (61/73, 84%) but in less than half of the PV patients (9/21, 43%). Of the patients tested for the presence of anti-DSG3, 4 out of 8 (50%) PV patients and 2 out of 46 (4.4%) PF patients were positive. These two PF patients (one from subgroup PFCLST and group 1; the other from subgroup PFSLST and group 4) presented both antibodies. Of the four PV patients positive for anti-DSG3, two were positive also for anti-DSG1, and two were negative. The four PV patients negative for anti-DSG3 were also negative for ani-DSG1.

The comparison among all subgroups showed significant differences of both anti-DSG1 ($p < 2.2 \times 10^{-16}$, Figure 1) and anti-DSG3 ($p = 0.0003$, Figure 2) levels. As only two PF patients were positive for anti-DSG3, one presenting lesions and the other not, PF patients were considered as a single group for statistical analysis concerning this autoantibody. Pairwise comparisons between groups are shown in Tables 1 and 2. For anti-

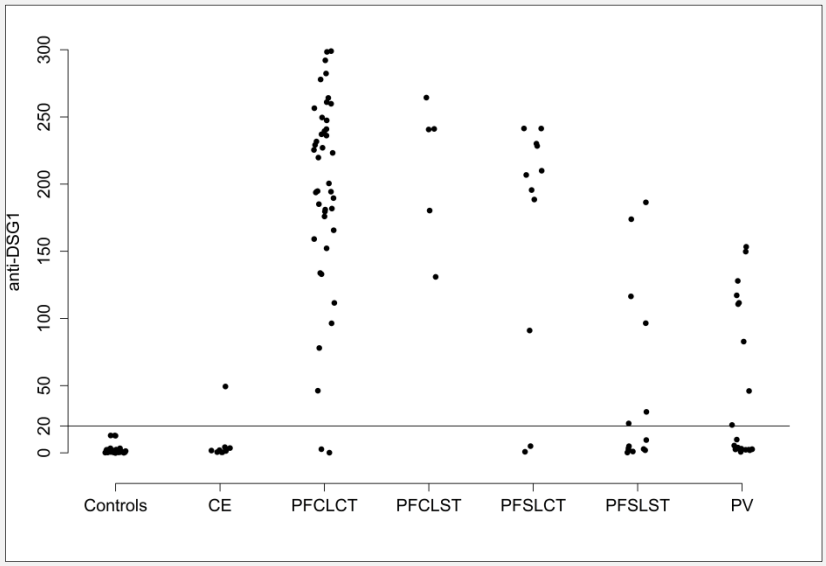

Figure 1. Levels of anti-DSG1 (U/mL) in pemphigus patients and control individuals.

Controls - clinically healthy individuals living outside the endemic region, CE - clinically healthy individuals that live in the endemic region, PFCLCT - patients presenting lesions and under immunosuppressive treatment, PFCLST - patients presenting lesions but not under immunosuppressive treatment, PFSLCT - patients not presenting lesions and under immunosuppressive treatment, PFSLST - patients not presenting lesions and not under immunosuppressive treatment. Comparison among groups: $p < 2.2 \times 10^{-16}$.

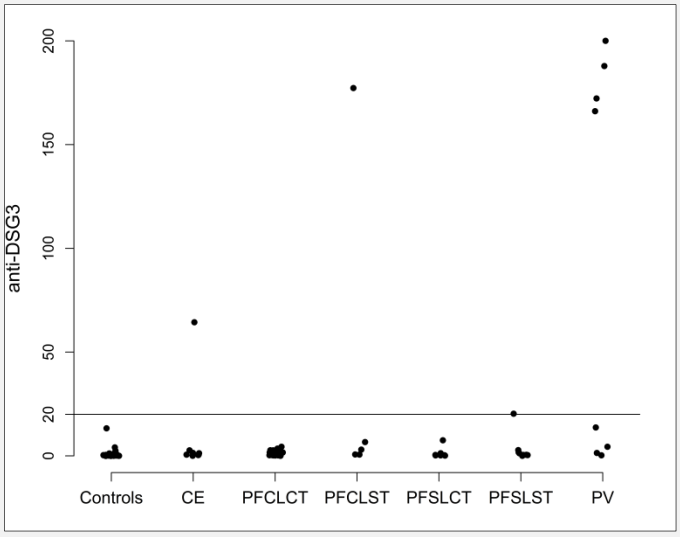

Figure 2. Levels of anti-DSG3 (U/mL) in pemphigus patients and control individuals.

Controls - clinically healthy individuals living outside the endemic region, CE - clinically healthy individuals that live in the endemic region, PFCLCT - patients presenting lesions and under immunosuppressive treatment, PFCLST - patients presenting lesions but not under immunosuppressive treatment, PFSLCT - patients not presenting lesions and under immunosuppressive treatment, PFSLST - patients not presenting lesions and not under immunosuppressive treatment. Comparison among groups: p = 0.0006.

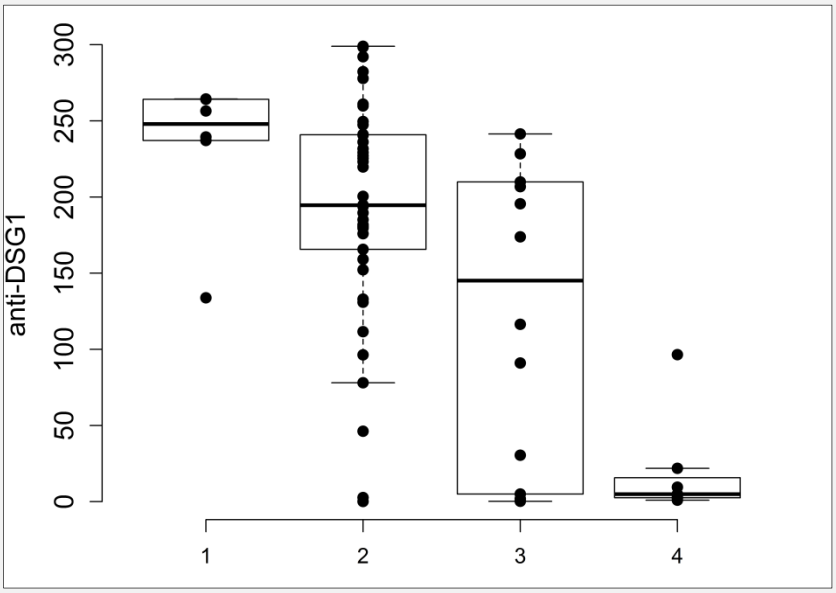

Figure 3. Levels of anti-DSG1 (U/mL) in PF patients classified according to severity of lesions or time without new lesions.

1) patients with many skin lesions, 2) patients with few isolated lesions, or lesions already under cicatrisation, 3) patients not presenting new lesions for a maximum of 18 months, 4) patients not presenting new lesions for more than 2 years. Comparison among groups: p = 0.0002.

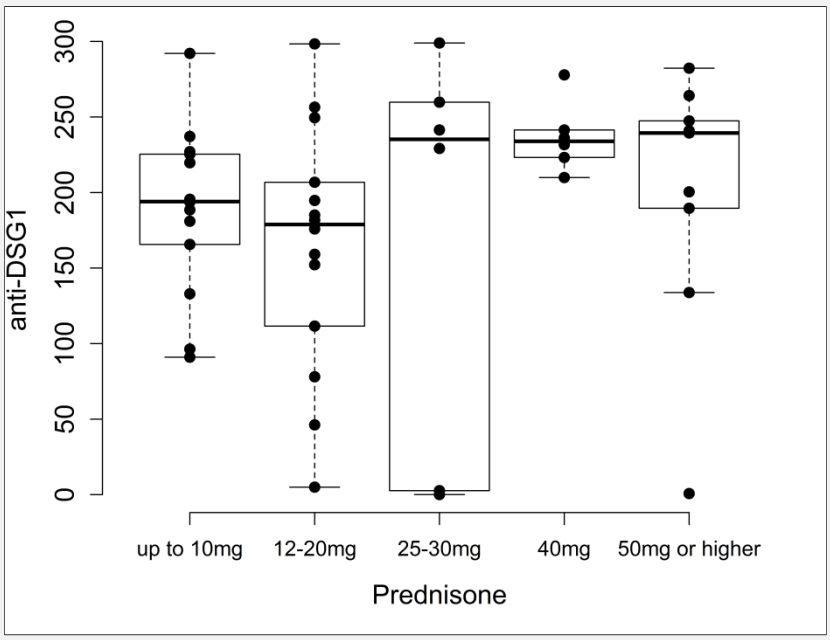

Figure 4. Levels of anti-DSG1 (U/mL) in PF patients classified according to the daily dose of immunosuppressive treatment.

Comparison among groups: p = 0.217.

DSG1, among PF subgroups, only the patients without lesions and not taking any immunosuppressive treatment (PFSLST) at the time of blood sampling differed from the other PF subgroups. The two subgroups of PF patients presenting lesions, either under immunosuppressive treatment (PFCLCT) or not (PFCLST), did not differ from each other nor from treated patients without lesions (PFSLCT). For anti-DSG3, PV patients differed from PF patients and from controls. Control individuals from endemic areas did not differ significantly from PF patients.

When comparing PF patients regarding the severity of lesions or time without lesions (Figure 3), a significant difference of anti-DSG1 levels was observed ($p = 0.0002$). Pairwise comparisons showed that patients with no lesions for a time shorter than 18 months (group 3 in Table 3) differed from patients with lesions (groups 1 and 2), but the difference between patients with few lesions or lesions already healing (group 2) was close to the significance limit ($p = 0.048$, Table 3) and may have occurred by chance.

Twenty PV patients were analyzed, but for three of them there was no information on the type of lesions (mucosal or cutaneous). For the other PV patients, there was a clear tendency of those with cutaneous lesions to present anti-DSG1 at higher levels (at least 80 U/mL). Only two individuals with mucosal lesions were positive for anti-DSG1, both with low levels (under 50 U/mL). Among the patients with mucocutaneous lesions, one was negative for anti-DSG1, but the other three presented high levels (above 80 U/mL). There were also four patients with only cutaneous lesions, two of which presented high levels of anti-DSG1 (above 110 U/mL). The other two were negative, but presented fewer lesions and were already under lower dosage of immunosuppressive treatment.

Usually, patients with a more severe disease receive higher doses of immunosuppressive treatment. The most common treatment delivered to pemphigus patients in Brazil is prednisone. Doses of prednisone were used to group patients, but no significant difference was observed among these groups regarding the levels of anti-DSG1 ($p = 0.5102$), even when only PF patients were considered ($p = 0.217$, Figure 4).

DISCUSSION

The individuals included in the present study were from a broad geographic area where PF is endemic, extending over the states of Mato Grosso do Sul, Mato Grosso, Minas Gerais, São Paulo, and Paraná. Previous analyses of the serum levels of anti-DSG1 and anti-DSG3 antibodies in Brazilian endemic PF patients were conducted in individuals living in Limão Verde, an Amerindian reservation area with the highest prevalence of PF ever reported (3.4%), and in the surrounding region [8,9]. Not surprisingly, anti-DSG3 and anti-DSG1 were more prevalent in PV and in PF, respectively. Anti-DSG1 levels differed significantly between PF and PV, between patients and healthy controls and between the subgroup of patients with no lesions and no immunosuppressive treatment (PFSLST) and other PF subgroups. Interestingly, this subgroup did not differ from the group of control individuals living in the endemic region (CE), although the p-value was close to the significance limit (0.055). When patients were classified by time without presenting new lesions, the CE subgroup did not differ from the group without lesions for two or more years (group 4). Therefore, the antibody profile of clinically healthy individuals from the endemic region is similar to the profile of patients in remission, especially in prolonged clinical remission, here defined as absence of new lesions for two years or longer. Patients with few lesions (group 2) were not that different for anti-DSG1 levels in serum from patients not presenting new lesions for a short period of time (group 3), who are in most cases still under immunosuppressive treatment. This last group is indeed very variable, as can be observed in Figure 3. This result reflects the difficulties of identifying active disease at this transition point, when patients are already recovering, but still at risk of relapsing.

The presence of autoantibodies in remission has been reported for endemic and sporadic PF [13-16]. Usually, remission is defined as just a few lesion-free months, or time is not even taken into account. Nonetheless, despite differences of methodology, all studies report a considerable number of patients remaining positive during remission. In the present work, of the 25 PF patients not presenting lesions, 15 (60%) were positive for anti-DSG1. If only patients already out of immunosuppressive treatment are considered, 5 (38.5%) were positive. Of the 7 patients without lesions for more than two years, 2 (28.6%) were positive. In Japan, Kwon et al. [14] observed 1 (16.7%) PF patient positive for anti-DSG1 in remission, when defining remission as no new lesions for at least 3 months and 5 mg/day or less of immunosuppressive treatment. However, we observed that patients in long-term remission are similar to the healthy individuals from the endemic region but differ significantly from patients with active disease. Because of significant physiological and immunological differences between patients in short time and in prolonged clinical remission, lesion-free and treatment-free time should be considered in future studies of immune responses after clinical remission.

A yet intriguing question is why anti-DSG levels remain positive in remission. As PV is less rare than PF worldwide, more studies are available for PV patients, where it has been shown that anti-DSG3 antibodies in remission are detected by conventional ELISA but not by conformational ELISA [17]. This indicates that the antibodies detected in remission may not be pathogenic. However, the same study did not find differences in conventional and conformational ELISA levels for anti-DSG1 antibodies. Additionally, several studies found that anti-DSG1 levels correlate with disease activity

better than anti-DSG3 levels, both for PV and PF patients [18,19]. For PF, Warren et al. [20] showed that IgG4 levels increase significantly more than other IgG subclasses during active disease; however, both healthy individuals from the endemic area and PF patients in remission still presented IgG4 anti-DSG1. Therefore, antibodies detected in patients in long-term remission, as well as those from control individuals, possibly are non-pathogenic. Alternatively, some patients in clinical short-term remission (no lesions for about 2 months) may still present circulating pathogenic autoantibodies. Follow-up studies including a large number of patients for a long period could help decide whether anti-DSG levels should be considered to establish when to withdraw the immunosuppressive treatment.

In the present study, 1 out of 9 (11%) healthy controls of the endemic region was positive for anti-DSG1 and anti-DSG3. The positive individual was born and lives in the state of São Paulo. The occurrence of anti-DSG1 autoantibodies in healthy individuals from São Paulo state was reported by Warren et al. [8], who found 5 (21%) positive individuals. In the surroundings of Limão Verde, 54 (19%) healthy individuals presented anti-DSG1 [8]. Hilario-Vargas et al. [9] observed anti-DGS3 antibodies in 8 (5.7%) healthy individuals in the area surrounding Limão Verde, but none of 18 healthy individuals from São Paulo state had anti-DSG3. In healthy individuals living close to an endemic focus of PF in Peru, 5 (12%) were positive for anti-DSG1; while in the endemic focus, 13 (32%) of the healthy individuals had anti-DSG1 [21]. Besides, anti-DSG antibodies have also been reported in 0.1% to 0.7% of random blood donors from non-endemic regions [22,23]. These results indicate that caution should be taken when using the autoantibodies for diagnosis, particularly for individuals living in the endemic region, or close to it. Pemphigus Foliaceus patients may also present anti-DSG3 autoantibodies, as was observed in the present work. Two out of the 46 (4.4%) tested PF patients were positive for anti-DSG3. One of them had high levels (above 170 U/mL) of both tested autoantibodies, while in the other both values were close to the cutoff level. This second patient was in remission and did not present new lesions for about 10 years. Arteaga et al. [24] observed anti-DSG3 in one out of 35 (2.9%) non-endemic PF and in 18 (7.5%) endemic PF patients. Li et al. [17] found PV patients with only cutaneous lesions that were positive for anti-DSG3 and suggested that it may happen because the oral mucosa has a higher expression of DSG3. For some cases of PV patients with anti-DSG3 antibodies but no mucosal lesions, these antibodies were shown to be less pathogenic [25]. On the other hand, occurrence of anti-DSG3 antibodies in PF patients is yet poorly understood. Nevertheless, the proportion of PF patients presenting anti-DSG3 is not negligible and the presence of this autoantibody is not recommended as the principal criterion for differential diagnosis. The presence of anti-DSG3 is highest in Limão Verde, where endemic PF reaches the startling prevalence of 3.4% and 9 of 21 (43%) patients had anti-DSG3 [9].

A high frequency of PV patients with cutaneous lesions presents anti-DSG1 antibodies [6]. We observed that PV patients with cutaneous or mucocutaneous lesions had a higher frequency (5 of 8, 62.5%) and higher levels (111 to 153 U/mL) of anti-DSG1 in comparison to those with only mucosal lesions (2 of 10, 20%; 21 and 46 U/mL), but three (37.5%) of them were negative. On the other hand, two of five PV patients with the disease localized to the mucous membranes were negative for anti-DSG3. Cases of PV patients with mucosal lesions only, that were negative for anti-DSG3, were previously reported [26]. In a similar situation, Kamiya et al. [27] used super concentrated sera and found positive results.

CONCLUSION

Almost all endemic PF patients presenting lesions had a positive ELISA test for anti-DSG1, but the same is not true for anti-DSG3 in PV patients. PF patients undergoing a more severe disease tend to have higher levels of anti-DSG1, but the levels are not significantly related to doses of immunosuppressive treatment. Furthermore, levels of anti-DSG1 are very variable among PF patients with few lesions or not presenting new lesions for a short time. Patients in long-term clinical remission (more than two years) are similar to healthy individuals of endemic areas for their autoantibody levels.

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Declaration of Interest:
None to declare.

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Supplementary Table 1. Anti-DSG1 and anti-DSG3 levels in serum of pemphigus patients and control individuals.

Controls not from endemic areas (Controls)								
ID	**Location**	**Age**	**Gender**	**Class**	**DSG1**	**DSG1_res**	**DSG3**	**DSG3_res**
C024JCM	UFPR	62	M		0.17	neg	-0.08	neg
C043MLP	UFPR	60	F		0.41	neg	0.00	neg
C068NBP	UFPR	58	F		0.59	neg		
C074KAB	UFPR	44	F		3.35	neg		
C096ALS	UFPR	42	F		12.87	neg	13.25	neg
C099ABB	UFPR	41	F		1.79	neg	0.32	neg
C100VSR	UFPR	56	F		0.30	neg		
C101VKC	UFPR	46	F		0.61	neg		
C134EFR	UFPR	56	F		0.35	neg		
C161TBO	UFPR	46	F		0.57	neg	0.16	neg
C287MRP	UFPR	33	F		1.25	neg	1.13	neg
C288DMF	UFPR	35	F		0.95	neg	0.25	neg
C331LAO	UFPR	32	F		3.32	neg	0.80	neg
C369GAC	UFPR	27	M		1.99	neg	0.09	neg
C384NMP	UFPR	63	M		0.35	neg	0.26	neg
C393ARS	UFPR	55	F		1.30	neg	0.09	neg
C409CFJ	UFPR	29	M		12.69	neg	0.00	neg
C410ERA	UFPR	43	M		0.23	neg	0.35	neg
C423AEV	UFPR	21	F		0.81	neg		
C423AEV	UFPR	20	F		-0.13	neg	009	neg
C428LTS	UFPR	27	M		2.39	neg	0.16	neg
C430LCP	UFPR	25	F		0.13	neg	-0.09	neg
C434ACB	UFPR	36	F		0.63	neg	0.26	neg
C439FSC	UFPR	21	M		0.25	neg	0.35	neg
C444MEK	UFPR	22	F		1.76	neg		
C449LCS	UFPR	21	F		0.23	neg		
C453CMC	UFPR	30	F		1.80	neg	0.79	neg
C464TDF	UFPR	28	F		0.70	neg	0.08	neg
C465JSJ	UFPR	21	F		0.51	neg		
C469MCM	UFPR	20	F		2.96	neg	4.04	neg
C472EKC	UFPR	29	M		1.40	neg	0.44	neg
C473IRR	UFPR	35	F		0.89	neg	2.38	neg
C474RHE	UFPR	34	M		0.00	neg	0.00	neg
C475VLC	UFPR	59	M		2.11	neg		
C476ICM	UFPR	55	F		0.51	neg	0.35	neg
C477OVO	UFPR	55	M		1.06	neg		
C478ZKA	UFPR	62	M		1.73	neg		
C479JOI	UFPR	30	M		12.90	neg		
C480LCO	UFPR	24	F		2.23	neg		

Controls from endemic areas (CE)								
ID	**Location**	**Age**	**Gender**	**Class**	**DSG1**	**DSG1_res**	**DSG3**	**DSG3_res**
C630ILB	HAP	74	F		0.38	neg	0.00	neg
C635AAT	HAP	68	M		0.38	neg	0.53	neg
C665HCF	HAP	33	M		4.19	neg	2.64	neg
C688SBA	HAP	43	M		3.52	neg		
C689EGB	HAP	26	M		1.65	neg	0.35	neg
C690AXA	FMRP	51	F		49.37	pos	64.35	pos
C691AXU	HAP	37	F		0.52	neg	0.57	neg
C692LHF	HAP	66	M		1.97	neg	1.21	neg
CP465NGP	HAP	46	M		1.40	neg	1.41	neg
PF patients without lesions and no immunosuppressive treatment (PFSLST)								
ID	**Location**	**Age**	**Gender**	**Class**	**DSG1**	**DSG1_res**	**DSG3**	**DSG3_res**
P242NRS	HAP	62	F	4	2.81	neg		
P307LRS	HAP	31	F	4	0.93	neg		
P360JIS	HAP	34	M	3	30.46	pos	1.23	neg
P362BCP (feb/14)	HFSU	53	F	NI	186.42	pos	2.73	neg
P376GOC	HFSU	52	F	3	1.90	neg	0.00	neg
P377IYY	HFSU	48	M	NI	3.40	neg		
P378YHP	HFSU	52	M	4	2.30	neg		
P380MQJ (nov/14)	HFSU	17	F	3	116.41	pos	0.48	neg
P387YLU	HFSU	30	M	4	96.47	pos	0.40	neg
P388MCY	HFSU	25	M	4	9.50	neg		
P413YNG	FMRP	19	M	3	0.20	neg		
P416OYX	FMRP	35	F	4	4.90	neg	0.32	neg
P417CQY	FMRP	63	M	4	21.87	pos	20.27	pos
PF patients without lesions and under immunosuppressive treatment (PFSLCT)								
ID	**Location**	**Age**	**Gender**	**Class**	**DSG1**	**DSG1_res**	**DSG3**	**DSG3_res**
P229MFF	HAP	52	F	NI	188.50	pos	0.40	neg
P266RMS	HAP	29	F	3	195.56	pos	0.18	neg
P352UDS	HAP	14	M	3	206.80	pos		
P356ADV	UFPR	37	M	3	173.86	pos	1.67	neg
P357MAF (2014)	UFPR	55	M	3	4.98	neg	0.26	neg
P363KHP (2014)	HFSU	13	F	NI	230.08	pos	0.26	neg
P367ABP	HAP	22	M	3	209.91	pos		
P369JCC	HAP	30	F	3	241.36	pos	1.23	neg
P382SCD	HAP	17	F	3	241.37	pos	0.18	neg
P384APK	HFSU	52	F	3	0.76	neg	7.48	neg
P392KEB	HAP	38	M	3	91.02	pos		
P419MVI	FMRP	24	F	3	228.36	pos	0.08	neg

PF with lesions and not under immunosuppressive treatment (PFCLST)								
ID	**Location**	**Age**	**Gender**	**Class**	**DSG1**	**DSG1_res**	**DSG3**	**DSG3_res**
P325CEA	HAP	48	M	2	241.05	pos	0.57	neg
P362BCP (nov/14)	HFSU	54	F	2	180.30	pos	6.62	neg
P391XQG	HFSU	15	F	1	264.35	pos	177.22	pos
P414AIK	FMRP	28	F	2	130.91	pos	0.65	neg
PK253EVM	HAP	62	F	2	240.61	pos	2.99	neg
PF patients with lesions and under immunosuppressive treatment (PFCLCT)								
ID	**Location**	**Age**	**Gender**	**Class**	**DSG1**	**DSG1_res**	**DSG3**	**DSG3_res**
P111JCM	HAP	40	F	2	236.10	pos	1.45	neg
P281FGS	HAP	47	F	2	200.46	pos	3.47	neg
P282NRA	HAP	32	F	2	298.94	pos		
P285JLO	HAP	31	F	2	282.30	pos	0.18	neg
P295EMS	HAP	34	F	2	194.76	pos	1.21	neg
P296ESA	HAP	54	F	2	249.54	pos		
P336ELC	HAP	33	F	2	132.92	pos	0.16	neg
P342GVN	HAP	56	F	1	264.13	pos		
P349CPP	HAP	35	F	2	259.77	pos		
P353JME	HAP	68	M	1	237.06	pos	0.00	neg
P357MAF (2011)	UFPR	52	M	2	78.00	pos	0.62	neg
P359RSG	HAP	25	F	2	111.57	pos		
P362BCP (may/14)	HFSU	54	F	2	179.57	pos	2.64	neg
P363KHP (2012)	HFSU	12	F	2	227.03	pos	0.26	neg
P364INS	HFSU	18	M	2	292.08	pos		
P366SSP	HAP	40	F	2	175.89	pos	2.46	neg
P368AOS	HAP	38	F	2	2.66	neg	1.50	neg
P370SOC	HAP	48	F	1	133.81	pos		
P371ASS	HAP	42	F	2	46.19	pos	0.26	neg
P372DPB	HAP	15	F	2	225.38	pos		
P373ITS	HAP	44	F	2	152.18	pos		
P374JBS	HAP	60	M	2	298.36	pos		
P375ACR	HAP	22	F	2	229.14	pos		
P379FPS	HFSU	25	F	2	260.94	pos	0.18	neg
P380MQJ (feb/14)	HFSU	16	F	2	165.61	pos	0.26	neg
P381VFS	HFSU	38	M	2	223.21	pos		
P383EYI	HAP	15	F	2	247.41	pos		
P385BXI	HFSU	12	M	2	277.87	pos		
P389DVC	HFSU	17	M	2	194.42	pos	4.36	neg
P390BLW	HFSU	32	F	2	159.11	pos	0.24	neg
P393OKQ	HAP	32	F	2	231.68	pos		
P394XSE	HAP	47	F	2	180.98	pos	2.67	neg
P395KEG	HAP	68	M	2	96.36	pos	1.62	neg
P396NMK	HFSU	31	F	1	239.41	pos	0.40	neg

P397KWV	HFSU	74	M	2	189.57	pos		
P398HKU	HFSU	49	F	2	194.31	pos	1.70	neg
P399LAF	HFSU	33	M	1	256.49	pos	1.62	neg
P407OEV	HAP	32	M	2	181.78	pos	0.73	neg
P408NKP	HFSU	29	M	2	193.74	pos	0.24	neg
P409JGF	HFSU	17	M	2	185.08	pos	0.32	neg
P411WDS	HFSU	32	M	2	240.89	pos	1.62	neg
P412YXZ	HFSU	47	F	2	219.70	pos		
P418KIP	FMRP	33	F	2	0.10	neg		
PV patients presenting lesions and under immunosuppressive treatment (PV)								
ID	Location	Age	Gender	Class	DSG1	DSG1_res	DSG3	DSG3_res
PV004JBA (2011)	HAP	35	F	m	20.71	pos		
PV004JBA (2013)	HAP	37	F	m	5.38	neg		
PV014VLT	UFPR	58	F	m	2.51	neg	1.37	neg
PV020DSF	UFPR	42	F	m	1.02	neg		
PV022SCL	UFPR	64	M	m	46.01	pos	172.21	pos
PV030SMC	HAP	80	F	c	9.85	neg		
PV034MCF	HFSU	80	F	m	2.34	neg		
PV035CGS	HFSU	33	F	mc	149.75	pos		
PV036EAL	HFSU	49	F	mc	3.05	neg		
PV037ASA	HAP	59	F	NI	111.61	pos		
PV038PDK	HAP	62	M	mc	110.66	pos		
PV039WKQ	HFSU	40	F	c	117.16	pos		
PV040EFD	HFSU	29	M	NI	127.92	pos	200.00	pos
PV043EPM	UFPR	34	M	m	1.90	neg	187.85	pos
PV046VYS	HFSU	48	F	mc	82.77	pos		
PV048CBN	UFPR	29	M	NI	0.68	neg	13.65	neg
PV049JNI	UFPR	38	F	m	2.62	neg	4.36	neg
PV056SQL	FMRP	31	F	m	2.73	neg	166.07	pos
PV059VSB	FMRP	45	M	c	2.23	neg	0.24	neg
PV060WQV	FMRP	38	F	c	153.30	pos		
PV061FFA	FMRP	41	F	m	3.86	neg		

When ID is followed by a date, it means more than one sample was available for the patient. PF - pemphigus foliaceus, PV - pemphigus vulgaris, Location - where samples were collected, Class - classification of patients according to severity of lesions, time without new lesions, or type of lesions (m - mucosal only, c - cutaneous only, mc - mucocutaneous), DSG1 - levels of anti-DSG1 (U/mL), DSG1_res - interpretation of the levels of anti-DSG1, DSG3 - levels of anti-DSG3 (U/mL), DSG3_res - interpretation of the levels of anti-DSG3, HAP - Hospital Adventista do Pênfigo, FMRP - Hospital de Clínicas da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, UFPR - Laboratório de Genética Molecular Humana (controls) or Hospital de Clínicas (patients) da Universidade Federal do Paraná, HFSU - Lar da Caridade Hospital do Fogo Selvagem de Uberaba, F - female, M - male, NI - no information, pos - positive, neg - negative.