Endemic Pemphigus Vulgaris

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Background: Investigators from Brasilia, Brazil, observed several patients with a mucocutaneous disease that resembles pemphigus vulgaris clinically and histologically but with epidemiological features of fogo selvagem. Our objective was to characterize antidesmoglein 3 and antidesmoglein 1 autoantibody profiles in these unique patients who reside in Goiânia and Brasilia, Brazil, known endemic regions of fogo selvagem.

Observations: We performed serological evaluation of 8 patients with a mucocutaneous disease clinically and histologically consistent with pemphigus vulgaris, as well as 27 healthy relatives of patients with fogo selvagem who reside in these endemic areas. Serum samples from all 8 patients bound desmoglein 3 by cold immunoprecipitation and from 6 patients by enzyme-linked immunosorbent assay, while serum samples from 4 patients bound desmoglein 1 by cold immunoprecipitation and by enzyme-linked immunosorbent assay. Antidesmoglein 3 autoantibodies were detected in 4 of 27 healthy donors by cold immunoprecipitation and by enzyme-linked immunosorbent assay, whereas antidesmoglein 1 autoantibodies were detected in 6 individuals by cold immunoprecipitation and in 3 individuals by enzyme-linked immunosorbent assay.

Conclusion: These findings provide serological evidence of a new endemic variant of pemphigus vulgaris.

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Pemphigus vulgaris (PV), pemphigus foliaceus (PF), and its endemic form fogo selvagem (FS) have distinct clinical, histological, epidemiological, and serological features. Pemphigus vulgaris is a disease characterized clinically by flaccid blisters or erosions involving the skin and mucous membranes and histologically by suprabasilar acantholysis. Patients with PV possess pathogenic antidesmoglein 1 (anti-Dsg1) and antidesmoglein 3 (anti-Dsg3) autoantibodies in its mucocutaneous form and solely anti-Dsg3 antibodies in its mucosal form.1-3 Most PV cases in North America, Europe, and Asia are sporadic, without evidence of geographic clustering. However, a few rare familial cases of PV have been reported.4-8 In addition, a slightly higher frequency of PV has been observed among the Ashkenazi Jewish population.9

In PF and FS superficial cutaneous blisters and erosions are seen clinically along with histological subcorneal acantholysis. Patients lack mucosal involvement. Serologically, pathogenic anti-Dsg1 autoantibodies are detected in patient serum.10-12 While PF and FS are identical clinically, histologically, and serologically,11 the epidemiological features of FS are distinctive. Fogo selvagem is a disease of peasants dedicated to outdoor activities, and cases exhibit geographic and familial clustering.14,15 These observations led investigators to suspect that the autoimmune response in FS is triggered by as yet unknown environmental factors.16-18 Other forms of endemic PF have been reported in Colombia, Peru, and Tunisia.19-22

Curiously, recent reports describe the rare transition of phenotype from PV to PF23-27 and from PF to PV.26,28,29 In addition, anti-Dsg1 autoantibodies, typical of PF, have been detected in serum samples from patients with PV.2 Conversely, anti-Dsg3 autoantibodies have been detected in serum samples from patients with PF and FS.30,31

During the past 3 decades, Brazilian investigators from the University of Brasilia have evaluated and treated many patients with FS at university clinics.32,33 Another group of investigators from Goiânia have treated several hundred patients with FS at the Hospital do Penfigo de Goiânia.14,34,35 Some of us (R.R.-A., H.F., and I.C.) have observed several patients with a mucocutaneous disease that resembles PV clinically and histologically but with epidemiological features of FS. Specifically, we observed the disease in younger patients residing in known endemic areas of FS. The objective of this study was to charac-
terize the anti-Dsg1 and anti-Dsg3 autoantibody responses in 8 of these patients initially seen and followed up at the University of Brasilia.

METHODS

PATIENTS AND SERUM SAMPLES

Between January 1, 1982, and September 30, 1989, serum samples were obtained from 8 patients with a mucocutaneous syndrome clinically and histologically resembling PV. Samples were kept frozen at −20 °C at the research laboratories of the University of Brasilia until they were transported to the Dermatology Research Laboratories, University of North Carolina at Chapel Hill. Serum samples from healthy donors in Brasilia (n=5) and in neighboring Goiás (n=22) were also included in the study. Figure 1 shows the geographic areas of interest in this study. The depicted regions are known to be endemic foci of FS. The sites of origin of the 8 patients are given in the Table. These sites represent rural communities surrounding urban Brasilia. Patients often travel long distances seeking medical care at the University of Brasilia Hospital. Serological studies were performed under University of North Carolina at Chapel Hill guidelines and institutional review board regulations.

PRODUCTION AND PURIFICATION OF RECOMBINANT Dsg1 AND Dsg3

Recombinant forms of Dsg1 and Dsg3 containing the entire extracellular domain and a C-terminal histidine tag were gener-
lated in the baculovirus system and were purified by nickel affinity chromatography. Purified recombinant Dsg1 and recombinant Dsg3 proteins were used in cold immunoprecipitation (IP) and enzyme-linked immunosorbent assay (ELISA) procedures.

**Dsg1 AND Dsg3 ELISA ASSAYS**

Recombinant Dsg1 or recombinant Dsg3 was immobilized on microtiter plates (Costar, Cambridge, Massachusetts) by overnight incubation at 4°C. The strips were then washed with a Tris-buffered saline solution, pH 7.4, containing 3.7 mM calcium. Duplicate samples of a 1:100 dilution of serum were incubated for 60 minutes. The plates were washed and then incubated with a 1:3000 dilution of horseradish peroxidase–labeled mouse antihuman IgG (Zymed Laboratories, South San Francisco, California) for 60 minutes. The strips were washed again and were incubated with o-phenylenediamine substrate (Sigma-Aldrich Inc, St Louis, Missouri) dissolved in phosphate citrate buffer with sodium perborate (Sigma-Aldrich Inc) for 30 minutes. The reaction was stopped with 4M sulfuric acid. ELISA values were expressed as an index value as previously reported by Amagai et al, using the following equation:

\[
\text{Index value} = \left( \frac{\text{test sample OD} - \text{negative control}}{\text{positive control OD} - \text{negative control}} \right) \times 100,
\]

where OD indicates optical density.

A cutoff value of 20 arbitrary units, previously determined by analyzing a set of 57 human serum samples from healthy donors in the United States, was used to separate positive from negative serum samples. Values below 20 were considered negative; those 20 and higher were considered positive. Well-characterized PV and FS serum samples were used as positive controls.

**INDIRECT IMMUNOFLOUORESCENCE AND COLD IP**

Indirect immunofluorescence (indirect IF) was carried out as previously described. Cold immunoprecipitation was performed using recombinant Dsg1 and recombinant Dsg3 as previously described. Skin and mucosal biopsy specimens were obtained from patients and were tested by hematoxylin-eosin staining. All serum samples from patients and healthy control subjects were tested by indirect IF using monkey esophagus as substrate according to previously published procedures.

**RESULTS**

Figure 2 shows the clinical features of 2 patients with mucocutaneous lesions similar to the mucocutaneous form of PV. Skin and mucosal biopsy specimens showed suprabasilar acantholysis (not shown). The findings from the serum samples of 8 patients by indirect IF, cold IP, and ELISA for Dsg1 and Dsg3 are given in the Table and in Figure 3. Autoantibodies against the epidermal intercellular spaces were detected by indirect IF analysis in all
patients, with titers ranging from 1:40 to 1:1280. Three of 27 serum samples (11%) from healthy controls tested positive by indirect IF, with titers ranging from 1:80 to 1:320. Serum samples from all 8 patients and from 4 of 27 control subjects (15%) immunoprecipitated recombinant Dsg3. Antidesmoglein 1 antibodies were detected by cold IP in 4 of 8 patients and in 6 of 27 control subjects (22%) (Figure 3). Antidesmoglein 3 autoantibodies were detected by ELISA in 6 of 8 patients and in 4 of 27 control subjects (15%). Antidesmoglein 1 antibodies were detected by ELISA in 4 of 8 patients and in 3 of 27 control subjects (11%). Positive and negative results from both tests used to detect specific anti-Dsg1 and anti-Dsg3 autoantibodies were concordant in 6 patients.

**COMMENT**

This study evaluated 8 individuals initially seen with a mucocutaneous syndrome resembling the mucocutaneous form of PV who also had autoantibodies against Dsg3 and Dsg1. The clinical features of these patients were unique, as FS does not involve mucous membranes. All 8 patients resided in rural communities surrounding Brasilia and Goiânia, well-known endemic areas of FS (Figure 1). No patients were from metropolitan Brasilia. Patients with FS treated at the University of Brasilia Hospital commonly travel from the communities of origin of the study patients. These clinical observations and the description of autoantibodies against Dsg3 in healthy individuals living in endemic areas of FS raised the suspicion that the findings in these patients may represent a rare form of endemic PV observed in these rural communities of Brazil where FS is also seen. Four of 8 patients were females younger than 20 years, an unusual finding in patients with PV. This observation may reflect hormonal effects in the onset and progression of autoimmune disease in these patients. Further studies are needed to clarify these aspects of the disease.

It is possible that this disease phenotype was previously overlooked because of its rare occurrence. For example, between 1952 and 1970, the Hospital do Penfigo de Goiânia, located in the center of an endemic region of FS, admitted 2663 patients with FS compared with only 37 patients with possible PV (ratio, 72:1). In the western regions of the state of Parana, another known endemic area of FS, Empinotti et al reported 213 FS cases and 11 PV cases (ratio, 19:1) between 1976 and 1988. Moreover, the Hospital Adventista do Penfigo in Campo Grande (state of Mato Grosso do Sul), Brazil, another hospital dedicated to the treatment of patients with FS, admitted 718 patients with FS and only 61 patients with PV (ratio, 12:1) between 1982 and 1988. In contrast, the University Hospital of Rio de Janeiro, Brazil, located in a nonendemic area, admitted 30 patients with FS and 14 patients with PV (ratio, 2:1) between 1959 and 1975. These admitted patients with FS had migrated from endemic regions of FS, whereas patients with PV resided within the city.

Antidesmoglein 1 antibodies have been detected in serum samples from most patients with FS and from one-third of healthy individuals living in the Limao Verde Reservation in Mato Grosso do Sul, Brazil, using ELISA. Autoantibodies against Dsg3 have also been detected in serum samples from 19 of 276 previously studied patients (7%) with FS and PF by ELISA. A recent study demonstrated anti-Dsg3 autoantibodies not only in 43% (9 of 21) of serum samples from patients with FS sera from the Limao Verde Reservation but also in 36% (53 of 146) of serum samples from healthy individuals living in and around this endemic area. A significant trend was observed in the proportion of positive test results for Dsg3 autoantibodies relative to distance from this endemic area. Despite this high observed prevalence of anti-Dsg3 autoantibodies among this population, no patients with FS or healthy individuals from this endemic area have displayed mucosal lesions suggestive of PV. In the present investigation, we detected anti-Dsg3 autoantibodies in the serum samples of 4 of 27 healthy individuals (15%) living in endemic areas of FS. Based on these findings, the emergence of pathogenic anti-Dsg3 autoantibodies and clinical variants of PV in the endemic regions of FS seems to be an uncommon occurrence. These results also reinforce the notion of potential environmental triggers relative to the emergence of these autoantibodies.

The lack of serological data before the development of disease in 8 patients presented herein limits our ability to more fully characterize the serological progression of this disease. However, given the observed seroepidemiological findings of anti-Dsg3 antibodies in residents of the Limao Verde Reservation, Brasilia, and Goiânia, it is conceivable that populations at risk of developing FS may also be at risk of developing an endemic form of PV. These findings underscore the importance of continued close clinical, serological, and epidemiological observation of populations living in endemic areas of FS.

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**Author Contributions:** Drs Rocha-Alvarez, Ortega-Loayza, Dasher, Li, and Diaz had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Rocha-Alvarez and Diaz. Analysis and interpretation of data: Rocha-Alvarez and Dasher. Drafting of the manuscript: Ortega-Loayza and Diaz. Critical revision of the manuscript for important intellectual content: Rocha-Alvarez, Ortega-Loayza, Aoki, Rivitti, Dasher, Li, and Diaz. Obtained funding: Li and Diaz. Administrative, technical, and material support: Ortega-Loayza and Dasher. Study supervision: Li and Diaz.

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