
Pemphigus foliaceus autoantibodies bind both epidermis and squamous mucosal epithelium, but tissue injury is detected only in the epidermis

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Background: Fogo selvagem (FS) is an autoimmune intraepidermal blistering disease mediated by antidesmosomal autoantibodies. Patients with FS do not have mucosal lesions despite extensive skin involvement.

Objective: Our purpose was to evaluate the epidermis and the oral epithelium of patients with FS as targets of antidesmosomal autoantibodies.

Methods: Fifteen patients were studied clinically, histologically, and immunologically. Biopsy specimens from the skin and the oral mucosa were studied by light microscopy and direct immunofluorescence. The serum of each of these patients was also titrated by indirect immunofluorescence.

Results: All patients showed skin lesions and subcorneal acantholysis, but none exhibited oral blisters or erosions. Direct immunofluorescence analysis demonstrated the presence of tissue-bound autoantibodies in both the epidermis and the oral epithelium of all patients with FS. Antiepidermal autoantibodies were also found in the sera of the patients.

Conclusion: Relevant epitopes on desmoglein 1 molecules of oral epithelium may not be available to react with pathogenic FS autoantibodies.

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Brazilian pemphigus foliaceus (PF), endemic PF, or fogo selvagem (FS) is an autoimmune epidermal disease characterized by subcorneal vesicles, autoantibodies against the desmosomal glycoprotein desmoglein 1 (Dsg1), and a distinct epidemiology.¹⁻³ Involvement of the skin varies in severity, that is, in some patients the vesicular eruption remains localized, whereas in others it becomes generalized, resulting in an exfoliative erythroderma.¹ Typically, involvement of the mucosal surfaces is not seen in patients with either of these two clinical forms of FS.

It is generally accepted that FS is similar to nonendemic PF clinically, histologically, and immunologically. FS autoantibodies are pathogenic and disease-specific; they are usually detected bound in vivo to the intercellular spaces (ICS) of lesional epidermis and circulating in the patient's serum.⁴⁻⁸ Furthermore, these autoantibodies are predominantly IgG^{4,9} and exhibit a degree of oligoclonality.¹⁰

The purpose of this investigation was to evaluate clinically and immunologically the epidermis and the oral mucosal epithelium of patients with FS as an initial step in understanding the absence of mucosal involvement in this disease.

MATERIAL AND METHODS

Patients with FS. Fifteen patients with FS at the Clínica de Ampollares del Hospital das Clínicas of the University of São Paulo were studied. The ages of these patients ranged from 11 to 68 years and included eight female and seven male patients of the following ethnic groups: seven whites, five mulattoes, and three blacks. All patients showed active epidermal disease with localized lesions in 14 cases and generalized disease in one (Fig. 1).

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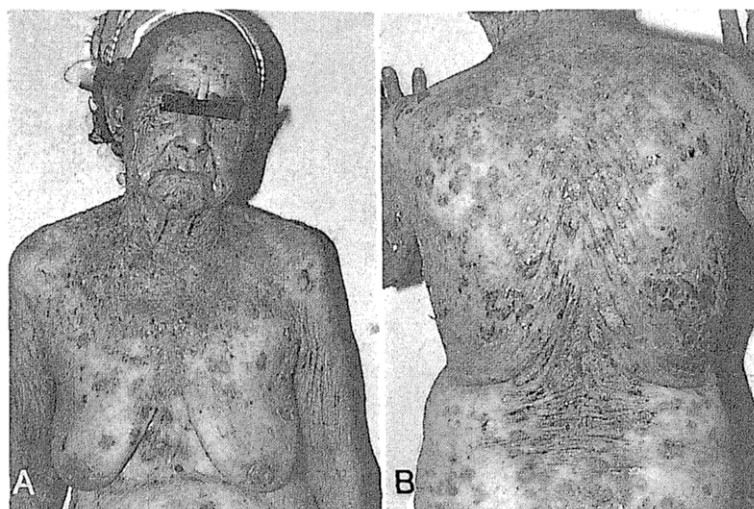


Fig. 1. Patient with generalized form of fogo selvagem shows extensive skin involvement. **A,** Face and chest. **B,** Trunk. No lesions were observed on the oral mucosa.

Table I. Immunofluorescence (IF)* studies of skin and oral mucosa in fogo selvagem

Case No.	Indirect IF (serum)	Direct IF (skin)					Direct IF (oral mucosa)				
		Whole IgG	IgG1	IgG2	IgG3	IgG4	Whole IgG	IgG1	IgG2	IgG3	IgG4
1	—	+	—	—	—	++	+	—	—	—	++
2	1:20	+	—	—	—	+	—	—	—	—	+
3	1:320	+	—	—	—	++	+	—	—	—	+
4	—	+	—	—	—	++	+	—	—	—	++
5	1:160	+	—	—	—	++	+	—	—	—	+
6	—	++	—	—	—	++	+	—	—	—	+
7	1:160	++	—	—	—	++	++	—	—	+	++
8	1:80	++	—	—	—	++	++	—	+	+	+++
9	1:80	++	—	—	—	++	+	—	—	—	++
10	1:160	++	—	—	—	++	+	—	—	—	++
11	1:40	++	—	—	—	++	+	—	—	—	++
12	—	++	—	—	—	++	+	—	—	—	++
13	1:80	++	—	—	—	++	+	—	—	—	++
14	—	+	—	—	—	++	++	+	—	—	++
15	1:40	+	—	—	—	++	++	+	—	—	++

*—; indicates negative IF test.

+; indicates increasing intensity of IF staining.

The studies were carried out in previously untreated patients (cases 1, 2, 9, 13, and 15) and in patients undergoing steroid therapy (cases 3, 4, 5, 6, 7, 10, 11, and 12). In two patients the steroid therapy was stopped by the patients before arriving at the clinic (cases 8 and 14).

Immunofluorescence studies. Biopsy specimens from perilesional skin and buccal mucosa were obtained with the patients under local anesthesia and processed for routine histologic examination and direct immunofluorescence evaluation. The serum of each patient was tested for IgG antiepidermal autoantibodies by indirect immunofluorescence procedures as reported elsewhere.⁹

RESULTS

As shown in Table I, there were two groups of patients with FS: The first group was composed of untreated patients (cases 1, 2, 9, 13, and 15) or patients who had stopped steroid therapy before coming to the clinic (cases 8 and 14). In this group the serum titers of FS autoantibodies by indirect immunofluorescence were below 1:80 ($n = 5$) or negative ($n = 2$). All patients in this group showed strong direct immunofluorescence staining of the epidermal and oral mucosal ICS (Figs. 2 and 3). The second

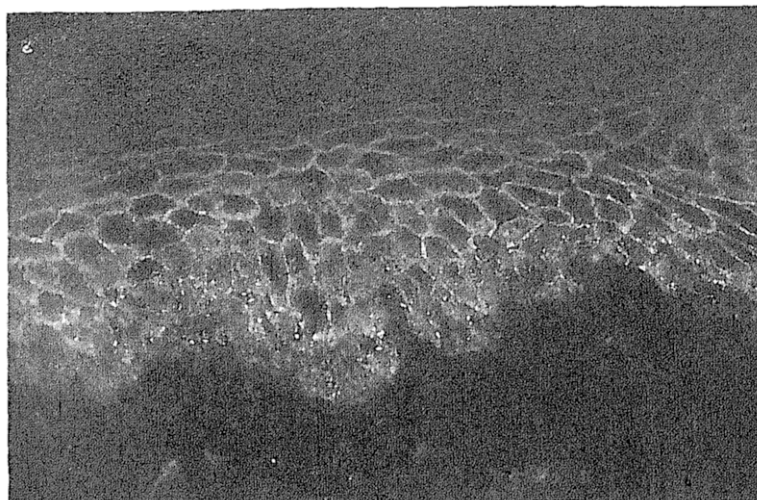


Fig. 2. Direct immunofluorescence examination of skin from patient with fogo selvagem. IgG4 autoantibodies stain the epidermal intercellular spaces. ($\times 400$.)

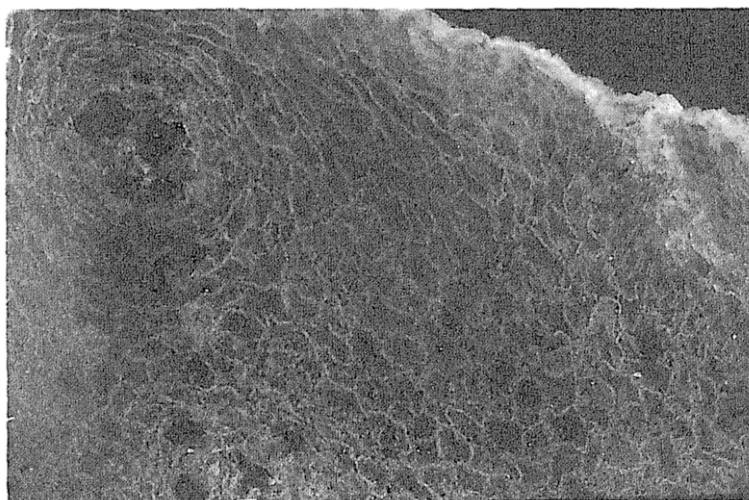


Fig. 3. Direct immunofluorescence of normal oral mucosa from patient with fogo selvagem exhibits staining of the intercellular spaces of the oral epithelium with IgG4 autoantibodies ($\times 400$.)

group included those patients undergoing steroid therapy (cases 3-7 and 10-12). In this group three patients had negative indirect immunofluorescence and five had serum titers above 1:40. This group also showed positive staining of the epidermal and mucosal ICS. All 15 patients with FS exhibited a positive direct immunofluorescence study of the epidermis and oral mucosa, even in patients in whom the serum was negative by indirect immunofluorescence. Although all 15 patients with FS in this study had skin lesions, none exhibited oral epithelial involvement, despite the fact that both squamous

epithelia demonstrated bound FS autoantibodies to the epithelial ICS.

DISCUSSION

Current experimental data strongly support the notion that FS autoantibodies are pathogenic and cause the induction of the epidermal lesions of FS. Whole IgG, IgG4, and Fab fragments of IgG from patients with FS induce epidermal blisters in neonatal mice by passive transfer experiments.^{8,9,11} In these experiments, however, the murine oral and esophageal mucosae were not studied. All patients

with FS included in this investigation exhibited different degrees of skin disease, but none of them had involvement of the oral epithelium. Because it is assumed that IgG4 autoantibodies in FS serum are pathogenic, it was surprising to detect IgG4 autoantibodies bound in vivo to the ICS of clinically uninvolved oral epithelium. FS autoantibodies of the IgG4 subclass were detected not only circulating in the serum of patients but also bound to diseased and uninvolved squamous epithelial tissues (i.e., the epidermis and the oral mucosa, respectively). Positive direct immunofluorescence of perilesional skin was always associated with a positive result in the patients' own normal mucosa. These results confirm and extend previous studies reported by Takahashi⁶ who found that the oral and esophageal mucosae of 28 patients with FS exhibited whole IgG bound to the ICS of these tissues, whereas intestinal biopsy specimens from the same patients showed negative results. Our investigation and Takahashi's study demonstrate that positive direct immunofluorescence of oral mucosal epithelium shows no correlation with either the titers of FS autoantibodies in the patient's serum or the clinical form of FS. These findings are in opposition to the well-established correlation between the titers of FS autoantibodies in the patient's serum and severity of the skin disease.⁷

The IgG autoantibody response in FS against Dsg1 is polyclonal and predominantly of the IgG4 and IgG1 subclasses.^{9,11} Furthermore, these autoantibodies appear to bind epitopes that localize to the extracellular region of epidermal Dsg1.¹²⁻¹⁴ Although the nature of the epidermal Dsg1 epitopes recognized by pathogenic FS autoantibodies was not determined by Allen,¹³ recent studies show that pathogenically relevant epitopes are localized on the N-terminal domain of Dsg1,¹⁴ the region of the molecule that is embedded in the epidermal ICS.¹² Therefore it is conceivable that in the epidermis these epitopes are accessible to pathogenic autoantibodies that permeate the intravascular space and percolate toward the epidermal ICS, where they cause injury. The same pathogenic autoantibodies may permeate the oral epithelium without causing injury because they do not find the respective epitopes on mucosal Dsg1. In vivo masking or absence of these epitopes on Dsg1 from oral epithelium are theoretical considerations. The latter possibility may suggest heterogeneity of the Dsg1 family of cadherins. It is expected that other nonpathogenic autoantibodies that are specific for other

epitopes of the extracellular regions of Dsg1 may also permeate the epidermal and mucosal ICS and produce positive direct immunofluorescence studies. It is also possible that the epidermal ICS and the oral epithelial ICS share other antigens that are recognized by irrelevant autoantibodies in the serum of patients with FS. These nonpathogenic autoantibodies could produce staining of the ICS of epidermis and oral epithelium by immunofluorescence techniques.

Finally, the structure and physiology of a keratinizing epidermis and nonkeratinizing oral epithelium may modulate the permeability of molecules moving through the epithelial ICS. This, in turn, may determine gradients of molecules, such as IgG at different levels of the epithelium. It is possible that the epithelial turnover and the absence of stratum corneum in the oral mucosal epithelium may also have implications in the immunopathogenesis of epithelial injury produced by FS autoantibodies.

It is anticipated that the specificity of pathogenic PF autoantibodies will be determined with molecular mapping techniques, shedding new light on the various hypotheses discussed here. Such an approach has been successfully applied to map pathogenic epitopes in the bullous pemphigoid antigen BP180 and was crucial in developing the first experimental mouse model of BP.¹⁵

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Computer image analysis in the diagnosis of melanoma

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Background: It is often difficult to differentiate early melanoma from benign pigmented lesions of similar clinical appearance.

Objective: Our purpose was to develop a computer image analysis system that has the potential for use as an adjunct to the clinical distinction of melanoma from less serious pigmented lesions.

Methods: The system, consisting of a hand-held device incorporating a color video camera and color frame grabber mounted in a microcomputer, was used in a pigmented lesion clinic. Analysis software extracted features relevant to the size, color, shape, and boundary of each lesion, and these features were correlated with clinical and histologic characteristics on which standard diagnoses of skin tumors are based. For discriminant analysis based on image analysis measurements, equal prior probabilities were assigned to two specified diagnostic groups, namely melanoma and "other pigmented lesions," most of which were melanocytic nevi.

Results: In a 20-month period, video images of 164 unselected pigmented lesions for which complete diagnostic data were available were successfully captured using the camera. Sixteen of 18 melanomas, and 89% of pigmented lesions overall, were correctly classified by the image analysis system, compared with 83% based on clinical gradings of lesion characteristics.

Conclusion: Computer image analysis has the potential to provide a valuable diagnostic aid that could enable clinicians to make highly sensitive and specific diagnoses of early, curable melanoma.

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There is increasing interest in the possibility that new imaging technologies may be used to enhance the diagnosis of cancer of various types. Cutaneous melanoma is unique among cancers in the sense that it is readily accessible and highly contrasted with the surrounding skin. Theoretically, computer imaging techniques could be applied in the diagnosis of melanoma directly and relatively easily. In comparison with other cancers such as breast cancer in which the