

Antigenic Specificity of Fogo Selvagem Autoantibodies Is Similar to North American Pemphigus Foliaceus and Distinct from Pemphigus Vulgaris Autoantibodies

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Fogo selvagem (FS) is clinically, histologically, and immunopathologically similar to sporadic pemphigus foliaceus (PF, as seen in North America and Europe), although the epidemiology of these 2 diseases differs markedly. It has been proposed that FS is identical to PF but, for some reason, occurs in an endemic focus in central Brazil. If this hypothesis is correct, the autoantibodies in FS and PF should have similar antigenic specificities. We studied sera from 13 patients with FS from central Brazil, and compared them with 20 sera from patients with PF from the United States. All these sera had circulating antibodies that bound the cell surface of epithelial cells in identical patterns by indirect immunofluorescence on monkey esophagus or normal human skin. In immunoprecipitation studies none of the 13 FS sera precipitated the pemphigus vulgaris (PV) antigen from radiolabeled extracts of cultured human keratinocytes. This is similar to the findings with PF sera of which 19 of 20 sera did not react with PV antigen, but in sharp contrast to the results with PV sera which, as previously

reported, all immunoprecipitate the PV antigen. Immunoblotting performed on extracts of normal human epidermis demonstrated that 7 of 20 PF sera specifically and intensely bound an approximately 160 kD polypeptide, previously identified as desmoglein I, a desmosomal glycoprotein. Similarly, 3 of 13 FS sera specifically bound a 160 kD polypeptide. Thirteen normal sera from North America, 8 normal and disease control sera from central Brazil, 11 PV sera, and 12 bullous pemphigoid sera did not specifically bind this polypeptide. Two-dimensional gel electrophoresis confirmed that the 160 kD polypeptides identified by the subgroup of PF and FS sera were identical. These studies demonstrate that, although the exact molecular specificities of the majority of PF and FS sera remain to be determined, FS autoantibodies do not bind the PV antigen and a subgroup of FS autoantibodies have molecular specificity identical to that of a subgroup of PF autoantibodies. *J Invest Dermatol* 87:197-201, 1986

Fogo selvagem (FS) is a type of pemphigus that occurs in an endemic focus predominantly in central and southern Brazil [1,2]. The clinical and histologic features of FS are similar to those of North American or European sporadic pemphigus foliaceus (PF). Both diseases are characterized by crusted and scaly lesions, often in a seborrheic distribution. Small flaccid bullae or pustules may also be seen in both diseases. Some cases of both FS and PF may begin as localized Senear-Usher syndrome. The pathology of both diseases demonstrates a subcorneal blister with acantholysis. Because of these clinical and histologic similarities to PF, FS has also been termed Brazilian or South American pemphigus foliaceus.

In addition to the aforementioned similarities, there are also identical immunohistologic findings in FS and PF. In both diseases

direct immunofluorescence demonstrates cell surface IgG throughout the epidermis, and indirect immunofluorescence, in most patients, demonstrates circulating IgG directed against the cell surface of normal stratified squamous epithelium [3]. Therefore, FS and PF are similar or identical diseases according to clinical, histologic, and immunohistologic criteria.

However, the epidemiology of FS differs markedly from that of PF [1,2]. FS occurs in an endemic focus whereas PF occurs sporadically. Furthermore, the age incidence of FS is different from that of PF. FS is more common in children, adolescents, and young adults with about one-third of cases occurring before 20 years of age [1]. There is also a high frequency of familial cases of FS (approximately 10% of cases), whereas familial PF is very rare. Thus, although very similar diseases when comparing individual patients, FS and PF differ dramatically in their epidemiology.

With these comparisons in mind, it has been proposed that the immunopathology of FS is identical to that of PF, but that, because of as yet unknown local factors, FS is more frequent and displays a unique epidemiology in Brazil. If this hypothesis is correct, then the autoantibodies found in both FS and PF patients should have identical antigenic specificities. Recent studies have begun to define the antigenic specificities, on a molecular level, of PF autoantibodies [4,5]. These studies have demonstrated that: (1) PF autoantibodies from different patients do not bind to the pemphigus vulgaris (PV) antigen, which is synthesized by keratinocytes in culture as a glycoprotein with disulfide-linked chains

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Abbreviations:

DG I: desmoglein I

FS: fogo selvagem

PAGE: polyacrylamide gel electrophoresis

PF: sporadic pemphigus foliaceus

PV: pemphigus vulgaris

SDS: sodium dodecyl sulfate

of 130 kD and 80 kD; (2) a subgroup of PF patients has antibodies that bind to a 160 kD glycoprotein extracted from normal human epidermis and identified by immunoblotting; and (3) this 160 kD glycoprotein, defined by these PF autoantibodies, is identical to desmoglein I (DG I), a desmosomal glycoprotein.

In this study we tested the sera from 13 patients with FS to determine whether the autoantibodies in this disease had antigenic specificities similar to those found with PF sera.

MATERIALS AND METHODS

Sera We studied the sera from 13 patients who were seen in the São Paulo Dermatology Department with clinically typical FS. Sera from 2 normals and 6 patients, with dermatologic disease other than FS, from the same area of Brazil were used as controls. The disease control patients were diagnosed as follows: leprosy (2 patients), psoriasis, paracoccidioidomycosis, vasculitis, and cutaneous tuberculosis. In addition, we studied the following sera from North America (some of which were included in previous reports [4,5]): 20 sera from patients with clinically and histologically confirmed PF, 11 sera from patients with clinically and histologically confirmed PV, 12 sera from bullous pemphigoid patients, and 13 normal sera.

Indirect Immunofluorescence Fogo selvagem, PF, and PV sera antibody titers to the cell surface of epithelial cells were determined by indirect immunofluorescence with normal human skin or monkey esophagus used as substrates [6].

Immunoprecipitation To determine antibody reactivity to PV antigen we used an immunoprecipitation procedure, as previously described in detail [4]. In brief, human keratinocytes, grown on irradiated 3T3 cells were metabolically radiolabeled with ^{14}C -labeled amino acids (NEC-445E, New England Nuclear, Boston, Massachusetts). Nonionic detergent (Nonidet P-40) extracts of these cells were preabsorbed with normal human serum and Protein A-bearing staphylococci (Pansorbin, Calbiochem-Behring Corp., San Diego, California). Preabsorbed radiolabeled extracts (about $2\text{--}4 \times 10^6$ cpm/immunoprecipitate) were then incubated with 5–10 μl of PV, PF, FS, or control sera, and the resultant immune complexes were precipitated with 50–100 μl of a 10% solution of Protein A-bearing staphylococci. Immunoprecipitates were solubilized with sample buffer [2% sodium dodecyl sulfate (SDS) in 62.5 mM Tris-HCl, pH 6.8 buffer] containing 0.1 M dithiothreitol, as a reducing agent. Radiolabeled precipitated proteins were then separated by SDS-polyacrylamide gel electrophoresis (PAGE), and specific bands were identified by fluorography.

Immunoblotting of Proteins Electrophoretically Separated in One and Two Dimensions To identify proteins that specifically bind antibodies in FS and PF sera, immunoblotting was performed on proteins extracted from normal human epidermis and separated by gel electrophoresis in 1 or 2 dimensions. These procedures have been previously described in detail [5,7]. Briefly, normal human epidermis was obtained either by excising the roofs (epidermis) of suction blisters induced on the forearm of normal volunteers or by 1 M NaCl-induced separation of the epidermis from the dermis of dermatomed normal human skin obtained from an amputated leg [8]. The epidermis was extracted with one of the following: sample buffer, sample buffer + 0.1 M dithiothreitol, or sample buffer + 0.1 M dithiothreitol + 8 M urea. These extractants also contained 1 mM phenylmethylsulfonyl-fluoride and 2 mM ethylenediaminetetraacetate. Proteins in these extracts were electrophoretically separated in one dimension by SDS-PAGE (under reducing conditions) or, in some experiments, by isoelectric focusing in one dimension, followed by SDS-PAGE in the second dimension [5]. The separated proteins, thus resolved in 1 or 2 dimensions, were electrophoretically transferred to nitrocellulose paper or 2-aminophenylthioether paper (which binds

proteins covalently) [7]. The proteins that reacted with antibodies in FS or PF sera were identified by immunoperoxidase staining [7].

RESULTS

Indirect Immunofluorescence Fogo selvagem and PF sera were titrated by indirect immunofluorescence with normal human skin or monkey esophagus as the substrate. All sera demonstrated IgG that bound the cell surface of epithelial cells at titers ranging from 40 to over 640 (Tables I, II). The patterns of cell surface staining of the FS and PF sera were identical. Although the titers of the FS and PF sera were variable, the subsequent immunoprecipitation and immunoblotting results were not correlated with the titer.

Antibodies in FS Sera Do Not Bind PV Antigen Cultured human keratinocytes were metabolically labeled with ^{14}C -amino acids, and the radiolabeled proteins were extracted with nonionic detergent. Sera from patients with PV consistently immunoprecipitated from these extracts a glycoprotein with disulfide-linked chains of 130 kD and 80 kD, termed here PV antigen [4] (Fig 1, lane 1). None of the 13 FS sera precipitated the PV antigen (Fig 1, Table II). Similarly, with one exception, the 20 PF sera also did not precipitate the PV antigen [4] (Table I). Thus, in sharp contrast to PV sera, both FS and PF sera, even those of high titer by indirect immunofluorescence, did not, in general, immunoprecipitate the PV antigen. In addition, neither PF nor FS sera specifically precipitated any other molecules from nonionic detergent extracts of these cultured keratinocytes.

Antibodies from a Subgroup of FS Patients Bind the Same Molecule as Antibodies from a Subgroup of PF Patients To try to identify the molecular specificity of FS antibodies, we performed immunoblotting using extracts of normal human epidermis. Polypeptides in epidermal extracts were separated by SDS-PAGE, then electrophoretically transferred to nitrocellulose or 2-aminophenylthioether paper. Identical lanes of protein were used for immunoperoxidase staining with FS, PF, or control sera.

Table I. Pemphigus Foliaceus Sera

No. ^a	Indirect Immunofluorescence Titer	Pemphigus Foliaceus Antigen	Pemphigus Vulgaris Antigen
		Immunoblotting Result (160 kD)	Immunoprecipitation Result (130, 80 kD)
96	320	— ^b	— ^c
175	320	+	—
177	80	+	—
223	>320	—	—
275	>640	+	+
276	40	—	—
297	320	—	—
442	40	—	—
444	40	+	—
457	160	+	—
458	160	—	—
463	160	—	—
517	160	—	ND ^d
520	160	+	—
566	40	—	—
682	80	—	—
684	>80	—	—
685	>80	—	—
686	>80	—	—
1092	640	+	—

^aDifferent numbers indicate sera from different patients.

^bMinus indicates no staining of 160 kD band; plus indicates staining on immunoblots of extracts of normal human epidermis.

^cMinus indicates that 130 and 80 kD bands were not precipitated; plus indicates that these bands were precipitated from radiolabeled extracts of cultured human epidermal cells.

^dNot done.

Table II. Fogo Selvagem Sera

No. ^a	Indirect Immunofluorescence Titer	Pemphigus Foliaceus Antigen	Pemphigus Vulgaris Antigen
		Immunoblotting Result (160 kD)	Immunoprecipitation Result (130, 80 kD)
567	>160	— ^b	— ^c
568	>160	+	—
569	>160	—	—
570	40	—	—
571	160	—	—
572	>160	—	—
573	>160	+	—
574	80	—	—
576	160	—	—
577	>160	—	—
578	160	—	—
586	>160	—	—
588	80	+	—

^aDifferent numbers indicate sera from different patients.

^bMinus indicates no staining of 160 kD band; plus indicates staining on immunoblots of extracts of normal human epidermis.

^cMinus indicates that 130 and 80 kD bands were not precipitated.

Three of the 13 FS sera specifically and intensely stained an approximately 160 kD polypeptide (Figs 2, 3; Table II). Although only 3 of the FS sera stained that band, the specificity of this staining was demonstrated for the following 2 reasons. (1) These FS sera intensely stained a very minor polypeptide band compared with the innumerable polypeptide bands transferred to the nitrocellulose, as shown in Fig 2. Lane AB in Fig 2 is a protein stain that demonstrates all the polypeptides, from the epidermal extract, that were transferred to the nitrocellulose strips. Lane FS 588 shows the immunoperoxidase stain of an identical lane of polypeptides with an FS serum. Of the total protein transferred to the nitrocellulose, the FS serum specifically stains only a minor band. (2) None of the many other control sera specifically stained this band. These controls included: 8 normal and disease (other than FS) sera from Brazil, 13 normal sera from North America, 11 PV sera, and 12 BP sera. Fig 2 demonstrates a typical immunoblot of an epidermal extract. The FS serum 588 (of indirect immunofluorescence titer 80) intensely stained the 160 kD band,

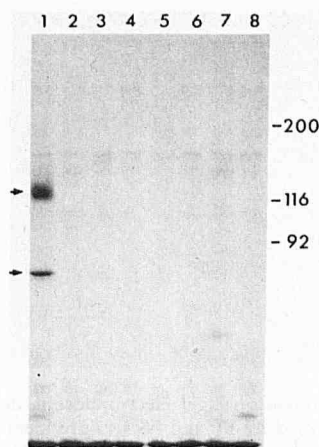


Figure 1. FS sera do not immunoprecipitate PV antigen. Immunoprecipitates of ¹⁴C-amino acid-labeled extracts of cultured normal human keratinocytes were separated by SDS-PAGE and radioactive polypeptides were identified by fluorography. Immunoprecipitations were performed with the following sera: lane 1, PV 443; lane 2, FS 567; lane 3, FS 573; lane 4, FS 572; lane 5, FS 588; lane 6, FS 586; lane 7, normal human serum 601; lane 8, normal human serum 563. Arrows indicate 130 kD and 80 kD bands of PV antigen. (Migration of molecular weight standards is shown at right, MW × 10⁻³).

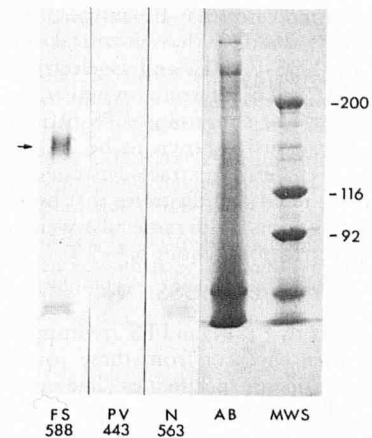


Figure 2. FS serum identifies a 160 kD polypeptide (arrow) on immunoblots of extracts of normal human epidermis. Extracts of normal human epidermis were subjected to SDS-PAGE and replicate lanes of the separated polypeptides were electrophoretically transferred to nitrocellulose. Immunoperoxidase staining of replicate lanes was performed with FS, PV, or normal human (N) serum. Different numbers indicate sera from different patients. AB indicates an amido black stain of all the polypeptide bands transferred to the nitrocellulose. MWS shows the molecular weight standards (MW × 10⁻³).

whereas the PV serum 443 (of indirect immunofluorescence titer over 320) and the normal human serum 563 did not. (Also see [4] for the specificity of staining.)

The band identified by this subgroup of FS sera comigrated on SDS-PAGE with the 160 kD band identified by a subgroup of PF sera [4] (Fig 3). Of 20 PF sera tested, 7 specifically bound to this 160 kD polypeptide. Therefore, both FS and PF sera give similar immunoblotting results in that a subgroup of each binds intensely to a 160 kD polypeptide extracted from normal human epidermis.

We have previously demonstrated that the 160 kD polypeptide identified by antibodies from this subgroup of PF patients is DG I, a desmosomal core glycoprotein [5]. Analysis by 2-dimensional

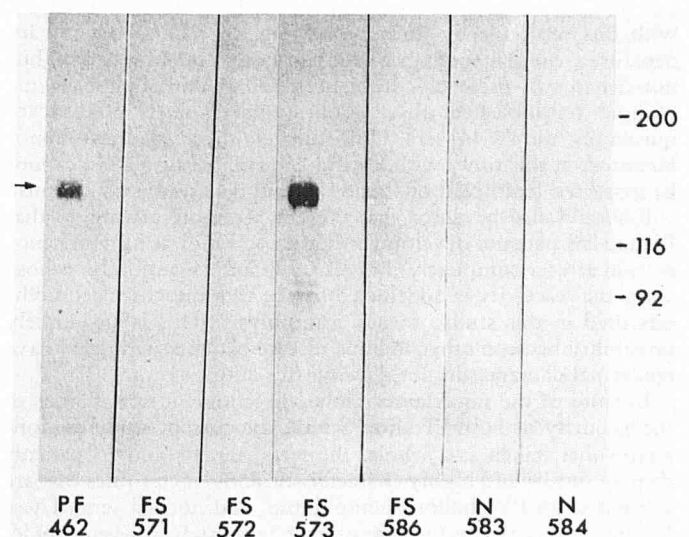


Figure 3. The 160 kD polypeptide (arrow) identified by certain FS sera on immunoblots comigrates with PF antigen. The same procedure was used as outlined in Fig 2, but on 2-aminophenylether paper. Immunoperoxidase staining was performed with PF, FS, or normal human (N) serum. Note that FS 573, but not the other FS sera shown here, stains the 160 kD band which comigrates with the band stained by the PF serum.

gel electrophoresis indicates that PF antigen, as defined by this subgroup of patients, and DG I have identical molecular weights, as determined by SDS-PAGE, and isoelectric points (pH approximately 5.4–5.7). To determine whether the 160 kD polypeptide identified by the subgroup of FS patients is identical to the PF antigen, previously shown to be DG I, we performed immunoblotting of epidermal extracts separated by 2-dimensional gel electrophoresis. Fig 4 demonstrates that both PF and FS sera identify comigrating spots, with molecular weight approximately 160,000 and isoelectric point about 5.4–5.7.

DISCUSSION

Our hypothesis was that if PF and FS are immunopathologically similar diseases then the sera from these patients should have similar molecular antigenic specificities. The antigenic specificities of PF sera are only partially known. Specifically, these sera do not, in general, bind PV antigen, and a subgroup of these sera bind DG I, a 160 kD desmosomal glycoprotein that can be extracted from normal human epidermis [4,5]. This study demonstrates that FS sera show similar antigenic specificities, with no binding to PV antigen and a subgroup that binds to the same molecule (DG I) to which the subgroup of PF sera bind. Even though only a minority of these patients' sera bind to this 160 kD molecule, this binding is highly specific for FS and PF; other disease control and normal sera do not display the same antigenic specificity.

It is important to note that the exact antigenic specificities (on a molecular level) of the majority of both PF and FS sera are not known. These sera do not precipitate any particular polypeptide from radiolabeled extracts of cultured human keratinocytes and do not bind any particular band on immunoblots of normal human epidermal extracts. The reasons for these findings are unclear, but there are several possible explanations. It is possible that PF, as well as FS, sera have different antigenic specificities, with a subgroup that binds DG I and the others directed against a different molecule that is difficult to identify by the immunochemical methods used here. Although this explanation might be feasible for a sporadic disease such as PF, it seems unlikely for an endemic disease such as FS. It seems more probable that in an endemic disease the immunopathology should be identical in different patients. Thus, a more likely explanation for the different immunochemical antigenic specificities of the FS (and, probably also the PF) sera is that they all react to the native DG I molecule, but that only a subgroup of sera retain their immunoreactivity with this molecule by immunoblotting, in which proteins are denatured. Similar findings of autoantibodies binding native, but not denatured, molecules have been found with bullous pemphigoid (unpublished observation), epidermolysis bullosa acquisita [8], and PV [4] sera. Unfortunately this hypothesis cannot be tested, at this time, with PF and FS sera, because DG I cannot be extracted from cells or tissue without denaturing conditions.

It should also be noted that there is a remote possibility that PF and FS patients develop antibodies to DG I as an epiphenomenon after a completely different cell surface antibody, whose antigenic reactivity is undetected by the immunochemical methods used in this study, causes acantholysis. This is an unlikely possibility because other bullous disease patients, who also have epidermal damage, do not develop this antibody.

In spite of the uncertainty about the antigenic specificities of the majority of both PF and FS sera, the current study demonstrates that, taken as a whole, the sera from PF and FS patients display similar and highly characteristic antigenic profiles that are distinct from PV, bullous pemphigoid, and normal sera. These findings support the hypothesis that, although different epidemiologically, the diseases PF and FS are similar immunopathologically.

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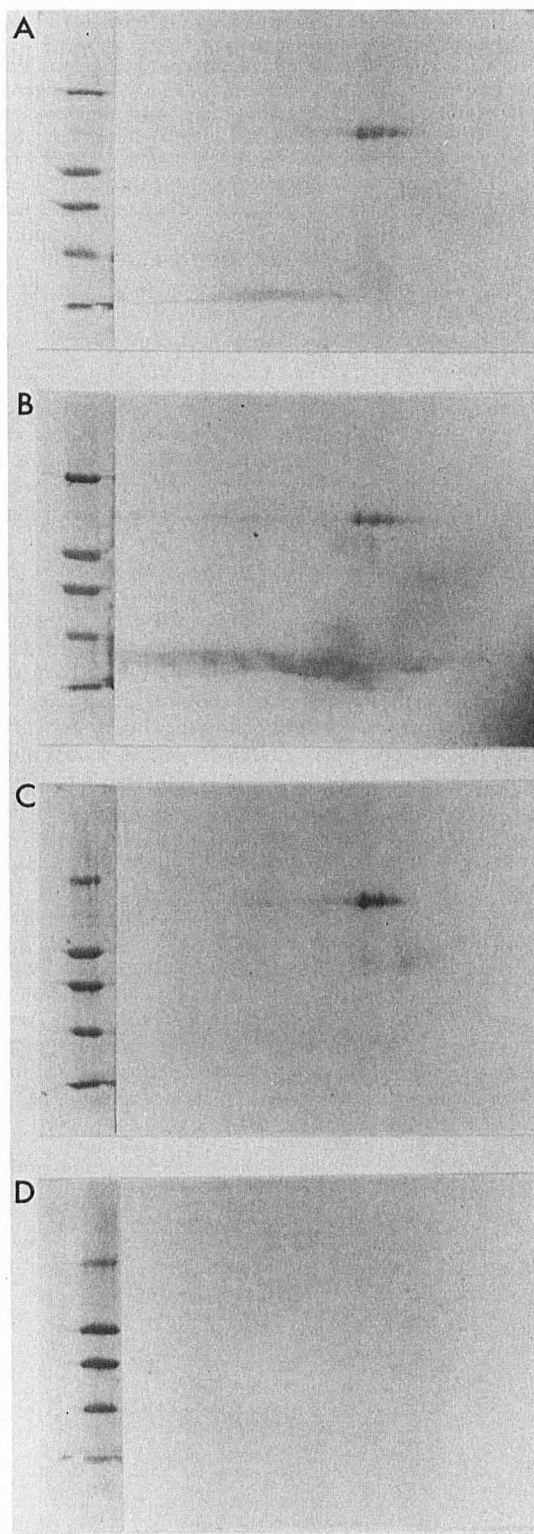


Figure 4. Two-dimensional gel electrophoresis demonstrates that the polypeptides identified by PF and FS sera are identical. Polypeptides in extracts of normal human epidermis were separated by isoelectric focusing in the horizontal dimension (acid end on right), then by SDS-PAGE in the vertical dimension. The polypeptides were then electrophoretically transferred to nitrocellulose sheets and subjected to immunoperoxidase staining with: A, PF 520; B, FS 588; C, FS 573; D, disease control serum from Brazil 584. Note comigrating spots in A, B, and C, with isoelectric points about 5.4–5.7 (determined by measuring the pH along a concurrently run isoelectric focused sample), and approximate MW 160,000 (as determined by molecular weight standards, shown in lane on left of each immunoblot, run by SDS-PAGE concurrently with second dimension—bands are 200 kD, 116 kD, 92 kD, and 68 kD).

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