Environmental Risk Factors in Endemic Pemphigus Foliaceus (Fogo Selvagem)

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An ongoing sero-epidemiological study of the Terena reservation of Limao Verde, known to have a high prevalence and incidence of FS, has revealed important information about this autoimmune disease. During surveillance of this population of approximately 1,200, which began in 1994, we documented 43 FS cases and studied the transition from the normal state to the disease state in several of these individuals. Furthermore, we established that FS patients as well as a large number of normal individuals on the reservation possess anti-dsg1 autoantibodies. The following interesting observations were made: (1) the ectodomain of dsg1 contains epitopes recognized by both autoantibodies and T cells from FS patients; (2) pathogenic anti-dsg1 autoantibodies in FS belong to the IgG4 subclass; (3) nonpathogenic anti-dsg1 autoantibodies of the IgG1 subclass were detected in normal individuals from Limao Verde and in patients in the preclinical stage of the disease; (4) anti-dsg1 autoantibodies from normal individuals and patients in the preclinical stage of FS recognize the EC5 domain of dsg1, whereas pathogenic anti-dsg1 autoantibodies bind the EC1/EC2 domains; (5) houses of FS patients are rustic, with thatched roofs and walls and dirt floors; (6) there was a high frequency of hematophagous insects (bedbugs and kissing bugs) in the houses of FS patients; (7) previous studies revealed that the predominant black fly on this reservation belongs to the species *Simunlium nigrimanum*. These findings suggest that the environmental antigen(s) triggering the autoimmune response in FS may be linked to exposure to hematophagous insects. ***Keywords: Pemphigus/Fogo Serlvagem/Desmoglein 1/Autoimmunity/Autoantibodies. J Investig Dermatol Symp Proc 9:34–40, 2004***

The endemic form of pemphigus foliaceus (PF) was reported in 1903 by Paes-Leme in Brazil (Paes-Leme, 1903). He described an epidemic of a unique blistering disease, thought to represent a clinical variant of tinea corporis (tinea imbricata or "tokelau"), in certain isolated regions of the state of São Paulo. Subsequently it was found (Vieira, 1937) that this endemic illness exhibited the same clinical features of PF reported by Cazenave in Paris in 1844 (Cazenave, 1844). In his classic monograph of 1937, Vieira established the salient clinical and histological features of this endemic form of PF, also known as fogo selvagem (FS) (Vieira, 1937). FS is distributed in impoverished rural areas of certain states of Brazil, where the disease is endemic (Paes-Leme, 1903; Vieira, 1937). A cutaneous disease with similar features to FS has been described in other countries, including Colombia and Tunisia (Robledo *et al*, 1988; Morini *et al*, 1993). FS is characterized by subcorneal epidermal blisters and autoantibodies against desmoglein 1 (dsg1) (Stanley *et al*, 1986; Diaz *et al*, 1989a). These autoantibodies are predominantly of the IgG4 subclass and are pathogenic, as demonstrated by passive transfer studies in neonatal mice (Roscoe *et al*, 1985; Rock *et al*, 1989).

Previous studies in FS documented its familial nature. For example, in a series of 2686 patients reported from the Hospital for Pemphigus in Goiania (Brazil), by Auad (Auad, 1972), 18% of cases were blood relatives, and 93% of these familial cases were found in genetically related family members. Recent studies have reported that the expression of DRB1*0404, 1402, or 1406 alleles is significantly linked to FS ($p < 0.005$, RR:14) (Moraes *et al*, 1997). The hypervariable region of the DRB1 gene of these alleles at the level of residues 67–74 shares the same sequence: LLEQRRAA. This shared epitope may confer susceptibility to FS, as is hypothesized for rheumatoid arthritis (Gregersen *et al*, 1987). FS affects people of many races and ethnic backgrounds, including Brazilians of Portuguese, Spanish, German, African, and Japanese descent who live in the endemic areas. Strikingly, the prevalence of FS in some states, for example, São Paulo, has decreased dramatically in recent years (Diaz *et al*, 1989b). It is thought that a local environmental agent or agents acting on genetically predisposed individuals in these communities might trigger a pathogenic anti-dsg1 antibody response that leads to FS. A hospital-based case–control study previously showed that *simuliid* bites were 4.7 times more frequent in individuals developing FS than in control individuals (Lombardi *et al*, 1992).

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Abbreviations: dsg1, desmoglein-1; FS, fogo selvagem; PF, pemphigus foliaceus; PV, pemphigus vulgaris.

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THE AUTOIMMUNE RESPONSE IN FS

Autoantibody response (Early Studies) It has been known for decades that the humoral antiepidermal autoimmune response in FS is mediated by IgG autoantibodies (Beutner *et al*, 1968; Rivitti *et al*, 1994). Moreover, it has been demonstrated that this IgG response is predominantly of the IgG4 subclass (Rock *et al*, 1989), although in some rare patients there is an IgG1 response exclusive of, or in combination with, IgG4 (Rock *et al*, 1989; Allen *et al*, 1993). Total IgG4, as well as the F(ab′)2 and Fab′ fragments of the FS IgG, is pathogenic in the mouse model of FS (Rock *et al*, 1990; España *et al*, 1997). Additional studies showed that the autoantibody response in FS exhibits a limited heterogeneity, consisting of oligoclonal IgG1 and IgG4 banding, when tested with epidermal antigens by affinity immunoblotting (Calvanico *et al*, 1993). These studies also showed that the autoantibodies in FS exhibit an early IgG1 response followed by a sustained IgG4 response. The results of this investigation appear to be in agreement with a clinical and serological study carried out in Minas Gerais, Brazil.[1] By indirect immunofluourescence (IF) techniques, this investigator found that the IgG1 autoantibody response in FS is present early in the course of the disease (patients showing skin blisters of less than six months of evolution). The IgG1 autoantibodies were undetectable in sera of patients entering the chronic phase of the disease (disease of more than six months of evolution) or when patients were effectively treated. IgG4 autoantibodies, however, were the predominant subclass in the sera of these patients.

It should be emphasized that human IgG4 antibody responses, as in FS, have been well documented in patients with filariasis (Ottesen *et al*, 1985), bee handlers chronically exposed to bee venom (Aalberse *et al*, 1983), and allergic individuals undergoing hyposensitization to environmental antigens (Oehling *et al*, 1998). In none of these patients, however, was the IgG4 response pathogenic as it is in FS.

The desmosome as the autoimmune target of pemphigus vulgaris and FS autoantibodies Stanley and colleagues (Eyre and Stanley, 1988; Stanley, 1989) demonstrated by immunoprecipitation techniques that the sera of patients with pemphigus foliaceus (PF) and pemphigus vulgaris (PV) recognize dsg1 and dsg3, respectively. These investigators also showed that FS sera recognize dsg1 (Stanley *et al*, 1986). Sequence analysis of dsg1 and dsg3 revealed that both antigens belong to the cadherin family of calcium-dependent cell adhesion molecules (CAMs) (**Fig 1**) (Goodwin *et al*, 1990; Koch *et al*, 1990; Amagai *et al*, 1991; Wheeler *et al*, 1991; Buxton *et al*, 1993). The desmosomal cadherins share extensive homology with other members of this gene superfamily of CAMs, such as desmocollins, and E-and p-cadherins (Amagai *et al*, 1991; Goodwin *et al*, 1990; Koch *et al*, 1990; Wheeler *et al*, 1991; Buxton *et al*, 1993). As in other members of this family, the extracellular regions of dsg1 and dsg3 are composed of five domains (EC1 to EC5). The first four domains are cadherin homologous repeats that contain six putative calcium-binding sites, and the fifth domain, EC5, is a short membrane-proximal region with no significant sequence homology to the other cadherin repeats. The intracellular domains are linked to the keratinocyte cytoskeleton via desmosomal plaque proteins. It is thought that homophilic interactions between ectodomains of desmoglein molecules and the interactions of these molecules with the intracellular cytoskeleton bring about epidermal cell–cell adhesion (Buxton *et al*, 1993). Interestingly, ablation of the dsg3 gene is associated with spontaneous erosions of the skin and mucosas and suprabasilar acantholysis as seen in PV lesions (Koch *et al*, 1997). These dsg3 knockouts show no anti-dsg3 autoantibodies, however. Amagai and colleagues (Amagai *et al*, 2000) used these dsg3 knockouts to study the pathogenesis of PV. They immunized mice with dsg3 and harvested their spleen cells, passively transferring them into SCID mice. The recipient animals developed anti-dsg3 antibodies and skin lesions typical of PV.

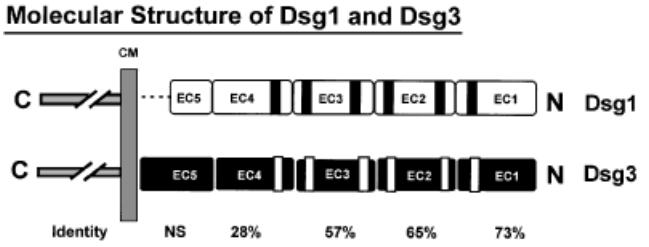

Figure 1. Molecular structure of the ectodomains of dsg1 and dsg3. dsg1 and dsg3 are two structurally similar transmembrane glycoproteins that belong to the cadherin family of calcium-dependent cell adhesion molecules. The extracellular domains of dsg1 and dsg3 contain four cadherin-like repeats (EC1 to EC4) and a membrane-proximal domain (EC5). The degree of sequence identity between these domains is also shown in this figure. In fogo selvagem (FS), the pathogenic autoimmune response is directed against epitopes localized in the EC1 and EC2 domains of the molecule. Non-pathogenic anti-dsg1 antibodies recognize epitopes on the EC5 domain and are present in normal individuals and FS patients during the preclinical stage of the disease.

T cells from FS patients also recognize desmoglein 1 We have shown that T cells from 13 of 15 patients with FS (Lin *et al*, 2000) recognize epitopes located on the dsg1 ectodomain. The proliferation of T cells from FS patients to dsg1 was antigen specific because they did not respond when incubated with other epidermal antigens, such as BP180. Moreover, T cells from control groups, including patients with BP, lupus, and psoriasis, as well as normal individuals, were unresponsive to dsg1. The response of these T cell clones was blocked by anti-DR antibodies, but not by anti-DQ or anti-DP antibodies, indicating that the dsg1-specific response of FS T cells is restricted to HLA-DR. The FS T cell clones expressed CD3, CD4, CD45RO, and TCR-$\alpha\beta$, but not CD8, CD19, or CD45RA, suggesting that they are CD4 memory T cells. The T cell clones derived from FS patients produce IL-4, IL-5, and IL-6, but not IFN-γ, indicative that they secrete a TH2-like cytokine. The type II cytokines, such as IL-4, might be relevant in modulating the IgG subclass response in these patients.

These findings strongly suggest that the ectodomain of these molecules is the target for both pathogenic autoantibodies and regulatory T cells. The epitopes recognized by FS autoantibodies and autoimmune T cells on dsg1 are the subject of intense investigation in different laboratories around the world.

DEFINING THE ENDEMIC FOCUS OF FS IN THE TERENA AMERINDIAN RESERVATION OF LIMAO VERDE, BRAZIL

We have described two settlements of Amerindian natives in Brazil that exhibit a high prevalence of FS: a Xavante Reservation located in the eastern region of the state of Mato Grosso (Friedman *et al*, 1995) and the Terena Reservation of Limao Verde (Hans-Filho *et al*, 1996). Since 1994, a clinical and serological surveillance of the Terena population of approximately 1200 individuals settled in Limao Verde has revealed a prevalence of FS of 3.4% and an incidence of one to four new cases per year (**Table 1**). With a highly specific and sensitive dsg1 ELISA, the presence of dsg1-specific autoantibodies was investigated, in the sera of FS

[1]Dos Santos S: Perfil evolutivo das subclasses de imunoglobulinas gama em pacientes de penfigo foliaceo endemico. Doctoral thesis, Universidade Federal do Rio de Janeiro, 1996

Table 1. The frequency of new FS cases on the Limao Verde Reservation (1994–2002). The population of this settlement has been relatively stable (~1,200 individuals). Twenty-two FS cases were documented before 1994 (Hans-Filho and *et al*, 1996)

Years of Surveillance	1994	1995	1996	1997	1998	1999	2000	2001	2002
Number of cases	4	1	1	2	2	1	1	5	4

patients and controls (Warren *et al*, 2000). Included in this investigation was a large group of normal donors from the USA, England, Japan. and Brazil. Also, the control sera from Brazil included samples from donors living in cities located at different distances from the Limao Verde reservation. As expected, FS patients from Limao Verde exhibited a positive dsg1 ELISA test. Anti-dsg1 autoantibodies were absent in control normal human sera from U.S., English, and Japanese donors (including 46 sera from U.S. Choctaw Indians) and from patients with other cutaneous autoimmune blistering diseases, such as bullous pemphigoid, herpes gestationis, and lupus erythematosus. Intriguingly, anti-dsg1 autoantibodies were detected not only in FS sera but also in sera of normal controls from the Limao Verde reservation (55% of 93 sera tested) and from inhabitants of other Brazilian cities (19% of 279 tested). The percentage of ELISA-positive sera among these normal control populations was inversely related to the distance from the endemic focus of Limao Verde (**Fig 2**). In five FS cases followed in Limao Verde for several years, anti-dsg1 autoantibodies were present in blood samples obtained one to five years prior to the onset of the disease. In this interesting subset, the titers of anti-dsg1 antibodies increased several fold once the disease was clinically apparent.

As seen in **Table 1**, the number of new FS cases in Limao Verde in recent years is high, indicating that this focus of disease remains active. These results suggest that certain members of the Limao Verde population become sensitized to an environmental antigen(s) producing anti-dsg1 autoantibodies that, in the course of several years, can lead to FS. Moreover, the risk of exposure to the putative environmental antigen(s) appears to be higher in Limao Verde than in other Brazilian populations distant from this region. It can therefore be speculated that (1) the environmental antigen(s) is unique and present in optimal amounts in Limao Verde; (2) the Terena population is highly susceptible to FS because of the inbreeding that is common in this settlement; or (c) both environment and genetic predisposition is relevant.

Although the molecular mechanisms of anti-dsg1 formation and the putative environmental antigen(s) in FS remain unknown, it is likely that epidermal dsg1 and the environmental antigen(s) share certain cross-reactive epitopes that are involved in FS pathogenesis.

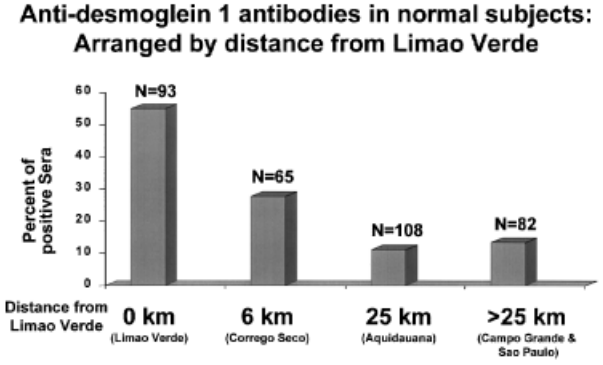

Figure 2. A greater percentage of normal individuals living on the Limao Verde reservation possess anti-dsg1 antibodies as compared with other Brazilian populations located at different distances from this endemic focus.

RECENT STUDIES ON THE IGG SUBCLASS ANTI-DSG1 RESPONSE IN FS IN LIMAO VERDE

We extended our sero-epidemiological studies of the Terena people of Limao Verde (Warren *et al*, 2000) by adapting the dsg1 ELISA to measure the subclasses of IgG anti-dsg1 autoantibodies present in FS patients and controls (Warren *et al*, 2003). We found that normal subjects living in the endemic area possess low levels of IgG1 and IgG4 anti-dsg1 autoantibodies, whereas FS patients have similar levels of IgG1 but a mean 19.3-fold higher IgG4 response. Additionally, FS patients in remission show weak IgG4 anti-dsg1 autoantibody titers, and a 74.3-fold higher IgG4 response is associated with active disease. Finally, in five patients from whom we had blood samples both before and after the onset of clinical disease, a mean 103-fold rise in IgG4 was associated with disease onset, but only a mean 3.45 rise in IgG1. These results suggest that the early antibody response in normal subjects living in Limao Verde and in patients before the onset of clinical disease is mainly IgG1. Acquisition of an IgG4 response is a key step in disease development.

These results support the notion that progression from a preclinical to a clinical phase of the disease, as well as the transition from disease in remission to active disease, is closely associated with subclass switching from IgG1 to IgG4 (Warren *et al*, 2003). Ongoing studies in our laboratory indicate that IgG1 subclass anti-dsg1 autoantibodies from a normal subject from Limao Verde (exhibiting a single IgG1 anti-dsg1 response) are incapable of inducing disease in neonatal mice in passive transfer experiments when injected with amounts as high as 15 mg/g body weight. In contrast, mice injected with IgG4 anti-dsg1 autoantibodies from an FS patient at the dose of 1.5 mg/g body weight develop extensive skin disease.

Finally, the results of our study of IgG subclasses of anti-dsg1 autoantibodies make it possible to identify a subgroup of normal subjects—namely, those with an increased level of IgG4 anti-dsg1 autoantibodies—who may be at a higher risk of developing clinical disease. Additionally, the level of IgG4 anti-dsg1 autoantibodies, as measured by IgG-subclass ELISA, may be one of the most sensitive indicators of clinical activity in patients with FS.

EVOLUTION OF THE ANTI-DGS1 AUTOIMMUNE RESPONSE IN INHABITANTS OF LIMAO VERDE BY EPITOPE ANALYSIS

The epitopes recognized by PF sera on dsg1 are conformation sensitive (Kowalczyk *et al*, 1995) and calcium dependent (Eyre and Stanley, 1987; Labib *et al*, 1991). Additionally, pathogenic anti-dsg1 autoantibodies target the ectodomain of dsg1 (Amagai *et al*, 1995; Emery *et al*, 1995) and recognize the N-terminal region of the molecule (Olague-Alcala *et al*, 1994; Sekiguchi *et al*, 2001). Using domain-swapped dsg1 and dsg3, we recently determined the epitope profiles of anti-dsg1 autoantibodies present in the sera of normal subjects living in Limao Verde and in FS patients in different phases of the disease (preclinical and clinical; remissions and relapses) (Li *et al*, 2003).

Our study showed that anti-dsg1 antibodies from normal individuals and from FS patients pre-onset recognize the EC5 domain of the molecule. This is a short domain (residues 453–496) of dsg1 that shows no significant homology to other domains (EC1 to EC4). Significantly, transition from a preclinical to a clinical stage of FS was accompanied by the emergence of auto-

antibodies specific for EC1 and EC2 domains (residues 1–108 and 109–221, respectively). Moreover, sera from FS patients with active disease recognize the EC1 and EC2 domains of the molecule, whereas in patients in remission the autoimmune response is restricted to the EC5 domain only. Disease relapses of FS are manifested by reappearance of anti-EC1/EC2 autoantibodies. These data demonstrate that the anti-dsg1 autoimmune response in FS is dynamic and that the epitope specificity of these autoantibodies defines the disease phenotype. More interestingly, these findings suggest that the anti-dsg1 autoimmune response in FS is initially raised against nonpathogenic epitopes located on the EC5 domain of the molecule and that the pathogenic anti-EC1/EC2 autoantibodies are generated later through the mechanism of intramolecular epitope spreading. Epitope spreading is a phenomenon in which new epitopes, within the same or a different molecule, are recognized over time by T cells or B cells from an original non-cross-reactive antigenic site (Vanderlugt and Miller, 1996; Mamula, 1998). This phenomenon has been well demonstrated in various experimentally induced or spontaneous animal models of autoimmunity at both the T cell and B cell levels (McCluskey *et al*, 1998). The evidence of epitope spreading in human autoimmune diseases is limited, however.

On the basis of current findings, we propose a two-phase model to encompass the pathogenetic mechanisms of FS. In the first phase, an environmental antigen(s) that bears sequence homology with the EC5 domain of dsg1 triggers an initial nonpathogenic antibody response to dsg1's EC5 domain. At this stage, individuals remain free of skin disease. In the second phase, intramolecular epitope spreading occurs in certain genetically susceptible individuals, which leads to the production of pathogenic anti-dsg1 antibodies against the EC1 and EC2 domains. These antibodies, in turn, bind to the EC1 and EC2 domains of dsg1 and induce skin blistering. The incubation time between the first and second phases of FS can last as long as seven years, as observed in one patient. The IgG subclass of the anti-dsg1 autoantibodies generated in the two phases is likely to be influenced by cytokines released during each stage of the immune response. The initial phase might be driven by TH0 or both TH1 and TH2 cytokines; the anti-dsg1 autoantibodies generated in this phase are therefore of the IgG1 and IgG4 subclass (Warren *et al*, 2003; Li *et al*, unpublished data). In the second phase, primed T cells are mainly TH2 type (Lin *et al*, 2000), which means that the pathogenic autoantibodies are predominantly of the IgG4 subclass (Rock *et al*, 1989; Warren *et al*, 2003). It appears that the EC1 of dsg1 or EC2 epitopes are the drivers of the pathogenicity of the anti-dsg1 autoantibodies rather than the IgG subclass, however. Indeed, there is a subset of nonendemic PF that exhibits IgG1 anti-dsg1 autoantibodies specific for the EC1 and EC2 domains, which are pathogenic when tested on the passive transfer mouse model.[2]

Our epitope-mapping studies make it possible to identify a subgroup of normal subjects who are at risk to develop FS: those possessing HLA class II susceptibility alleles and carrying anti-dsg1 EC5 autoantibodies. Additionally, measuring the epitope specificities of anti-dsg1 autoantibodies may be the most sensitive indicator of clinical remission and relapse in FS patients. Mapping the initial epitopes located on the EC5 domain of dsg1 will be valuable as well in identifying the environmental etiology of FS.

DEFINING THE LOCAL ENVIRONMENT SURROUNDING FS PATIENTS IN LIMAO VERDE

A population-based case–control study of FS was conducted among residents of the Limao Verde reservation from July 1997 to October 2000. The majority of the residents living on this reservation are Amerindians of the Terena tribe. The rest of the population comprises non-Terena Amerindians from other ancestors (Bolivians, Xavantes, etc.) and individuals from other ethnic backgrounds (blacks, whites, and mestizos) A single investigator (VA) conducted the interviews and recorded the data with the assistance of a local community leader. Interviews were conducted in Portuguese, the native language of both the interviewer and the interviewees, and lasted approximately 40 minutes each.

All prevalent and incident FS patients in Limao Verde were interviewed (n = 30), including patients from the Limao Verde (n = 28) and Ipegue (n = 2) reservations. Community (n = 108) and family (n = 90) controls were selected by matching on age, gender, race, and occupation to the FS residents of Limao Verde. All controls were identified at the same time that the cases were interviewed. Community controls were chosen by randomly selecting houses to be visited within the Limao Verde reservation and consisted of persons with no blood relationship to the FS patients. Family controls were selected from among blood relatives of FS patients living in the Limao Verde reservation.

A 13-page questionnaire was administered to each FS patient and the selected controls. Demographic information, present and previous housing, characteristics of current residence (house construction, roof, walls, windows, floors, bedrooms, waste disposal, and water supply), occupation and other activities, dietary habits, exposure to blood-feeding arthropods at home and at work, and presence of domestic or wild animals around the house or at work were recorded. The interviewer made a final assessment of the house and living conditions of patients and controls. The collected information from both groups was entered into an Excel spreadsheet. Statistical analyses were conducted using SAS software (Cary NC) and consisted of separate tabulations for cases and the two control groups. Because of the complex sampling scheme for controls, no tests were conducted for statistical significance, and data are presented in tabular form for descriptive purposes only.

Results of the case–control study are presented in **Tables 2** through **5**. **Table 2** shows the demographic characteristics of FS patients and controls. The mean ages of surveyed residents were 40, 40.3, and 36.6 years for FS patients, community controls, and family controls, respectively. There were 17 male and 13 female FS

Table 2. Demographic characteristics of surveyed residents of Limo Verde and Ipegue

Characteristic	Cases (N = 30)	Community Controls (N = 108)	Family Controls (N = 90)
Age at interview (years)			
Mean (SD)	40.0 (14.9)	40.3 (14.6)	34.6 (18.9)
Median	42.0	40.0	30.5
Range	15–69	14–75	10–86
Gender			
Male	17 (56.7%)	62 (57.4%)	39 (43.3%)
Female	13 (43.3%)	46 (42.6%)	51 (56.7%)
Race/ethnicity			
Terena	22 (73.4%)	84 (77.8%)	63 (70.0%)
Other Indian	1 (3.3%)	3 (2.8%)	1 (1.1%)
White/Indian	7 (23.3%)	16 (14.8%)	23 (25.6%)
Black/Indian	0	5 (4.6%)	3 (3.3%)
Place of residence			
Limao Verde	28 (93.3%)	108 (100%)	90 (100%)
Ipegue	2 (6.7%)	0 (0%)	0 (0%)
Occupation			
Farming	20 (66.7%)	67 (62.0%)	34 (37.8%)
Tree/shrub removal	17 (56.7%)	56 (51.96)	32 (35.6%)
Fishing & Hunting	0 (0%)	5 (4.6%)	0 (0%)
Housework	9 (30.0%)	24 (22.2%)	30 (33.3%)
Other occupations	10 (3.3%)	41 (38.0%)	56 (62.2%)

[2]Li N, Liu Z, Diaz LA: Pemphigus foliaceus autoantibodies recognize two dominant pathogenic epitopes located in EC1 and EC2 domains of desmoglein-1. *J Invest Dermatol* 119:305, abstract, 2002

Table 3A. Housing characteristics of study participants

Characteristic	Cases (N = 30)	Community Controls (N = 108)	Family Controls (N = 90)
Electricity			
Yes	10 (33.3%)	54 (50%)	38 (42.2%)
No	20 (66.7%)	54 (50%)	52 (57.8%)
Indoor plumbing			
Yes	0	8 (7.4%)	2 (2.2%)
No	30 (100%)	100 (92.6%)	88 (97.8%)
Dirt floors			
Yes	26 (86.7%)	79 (73.2%)	71 (78.9%)
No	4 (13.3%)	29 (26.9%)	19 (21.1%)
Brick walls			
Yes	6 (20.0%)	45 (41.7%)	37 (41.1%)
No	24 (80.0%)	63 (58.3%)	53 (58.9%)
Adobe walls			
Yes	19 (63.3%)	45 (41.7%)	49 (54.4%)
No	11 (36.7%)	63 (58.3%)	41 (45.6%)
Thatched walls			
Yes	7 (23.3%)	15 (13.9%)	13 (14.4%)
No	23 (76.7%)	93 (86.1%)	77 (85.6%)
Thatched roof			
Yes	23 (76.7%)	44 (40.7%)	36 (40.0%)
No	7 (23.3%)	64 (59.3%)	54 (60.0%)
Tile roof			
Yes	11 (36.7%)	62 (57.4%)	60 (66.7%)
No	19 (63.3%)	46 (42.6%)	30 (33.3%)
Fitted doorway			
Yes	4 (13.3%)	29 (26.9%)	23 (25.6%)
No	26 (86.7%)	79 (73.2%)	67 (74.4%)
Outer door exists			
Yes	11 (36.6%)	68 (62.9%)	59 (65.5%)
No	19 (63.3%)	40 (37.0%)	31 (34.4%)

Table 3B. Other housing characteristics of study participants

Characteristic	Cases (N = 30)	Community Controls (N = 108)	Family Controls (N = 90)
Water supply piped from river (for cooking)			
Yes	21 (70.0%)	82 (75.9%)	67 (74.4%)
No	9 (30.0%)	26 (24.1%)	23 (25.6%)
Water supply piped from river (for laundry)			
Yes	18 (60.0%)	75 (69.4%)	57 (63.3%)
No	12 (40.0%)	33 (30.6%)	33 (36.7%)
Water supply piped from river (for bathing)			
Yes	19 (63.3%)	79 (73.2%)	58 (64.4%)
No	11 (36.7%)	29 (26.8%)	32 (35.6%)
Bathing at the river			
Yes	13 (43.3%)	32 (29.6%)	36 (40.0%)
No	17 (56.7%)	76 (70.4%)	54 (60.0%)
Laundry at the river			
Yes	13 (43.3%)	40 (37.0%)	49 (54.4%)
No	17 (56.7%)	68 (63.0%)	41 (45.6%)
Privy (toilet)			
None	4 (13.3%)	17 (15.7%)	16 (17.8%)
In the house	0 (0.0%)	1 (0.9%)	0 (0.0%)
Outside the house	26 (86.7%)	90 (83.3%)	74 (82.2)
Toilet (fields & bushes)			
Yes	4 (13.3%)	8 (7.4%)	12 (13.3%)
No	26 (86.7%)	100 (92.6%)	78 (86.7%)
Bed location			
On floor	0 (0.0%)	1 (0.9%)	0 (0.0%)
On raised platform	30 (100%)	107 (99.1%)	89 (100%)
Bed platform			
Bamboo	2 (6.7%)	7 (6.5%)	3 (3.4%)
Wooden boards	25 (83.3%)	90 (84.1%)	80 (90.9%)
Mat or hammock	1 (3.3)	3 (2.8%)	3 (3.4%)
Bamboo & wooden board	1 (3.3%)	5 (4.7%)	2 (2.3%)
Wooden board & other	1 (3.3%)	2 (1.9%)	0 (0.0%)
Mattress			
Open foam cushion	26 (86.7%)	92 (86.0%)	81 (91.0%)
Straw or woven mat	1 (3.3%)	6 (5.6%)	3 (3.4%)
Cloth mattress	0 (0.0%)	1 (0.9%)	0 (0.0%)
Hammock	2 (6.7%)	3 (2.8%)	3 (3.4%)
Open foam & straws	0 (0.0%)	3 (2.8%)	1 (1.1%)
Straw & cloth mat	1 (3.3%)	2 (1.9%)	0 (0.0%)
Open foam & cloth	0 (0.0%)	0 (0.0%)	1 (1.1%)
Mattress rests on platform			
Yes	19 (63.3%)	51 (47.2%)	50 (55.5%)
No	11 (36.6%)	57 (52.7%)	40 (44.4%)
Bedding exposed to sun			
Daily	0 (0.0%)	1 (0.9%)	1 (1.1%)
Occasionally	20 (66.6%)	62 (57.4%)	56 (62.2%)
Rarely or never	10 (33.3%)	45 (41.6%)	33 (36.6%)
Holes and cracks in bedding			
Yes	23 (76.7%)	59 (54.6%)	42 (46.7%)
No	7 (23.3%)	49 (45.4%)	48 (53.3%)
Bedbugs found in bedding			
Yes	20 (66.7%)	51 (47.2%)	36 (40.0%)
No	10 (33.3%)	57 (52.8%)	54 (60.0%)
Cleanliness of house (determined by interviewer)			
Very good or good	2 (6.6%)	10 (9.4%)	11 (12.2%)
Average	8 (26.7%)	39 (36.5%)	34 (37.8%)
Poor	11 (36.7%)	41 (38.3%)	40 (44.4%)
Very poor	9 (30.0%)	17 (15.9%)	5 (5.6%)
Missing	0	1	0

patients. Over 70% of study participants belonged to the Terena ethnic group that live in Limao Verde. Over 60% of FS patients and community controls engaged in farming activities, and over 50% of FS patients and community controls reported being involved in tree or shrub removal. A smaller proportion of family controls were involved in these activities.

Table 3A and **Table 3B** show the housing characteristics of the participants in the study. The salient features of the FS patients' housing are shown in **Fig 3**. More often than not, these houses contained dirt floors, adobe or thatch walls, thatched roofs, poorly fitted or nonexistent doors, and no indoor plumbing or electricity compared to community or family controls. The water supply used for cooking, bathing, and laundry was similar among FS patients and the two control groups. Poor toilet facilities were a common feature in all three groups, and characteristics of sleeping quarters were similar. The majority of individuals slept on a raised platform covered by a variety of bedding material. Interestingly, the interviewer identified bedbugs in 66.6% of the beds of FS patients versus 47.2% of community and 40% of family controls. The housing conditions of the FS patients were usually poor or very poor as judged by the interviewer.

Table 4 shows animals and insects found in or around the homes of participants. The participants possessed dogs, cats, and chickens and were exposed to rats, cockroaches, bedbugs (*Cimex* sp.), and kissing bugs (*Reduviid* sp.) that were usually identified by the interviewee and interviewer. Bedbugs and kissing bugs were detected more often in the homes of FS patients compared with community and family controls.

Table 5 presents bites reported by study participants from hematophagous bug bites during several months prior to interview (black flies, kissing bugs, bedbugs, mosquitoes, ticks, and

Figure 3. The house of a patient with fogo selvagem. A patient with FS (FS-1) is also shown. The housing characteristics are similar to those described as occupied by patients with Chagas disease (Despommier 1995, Prata, 1999). Chagas disease however, is unknown in Limao Verde.

fleas) and exposure to rodents, cereal powders, and a variety of domestic animals. FS patients reported a higher frequency than did controls of black fly, kissing bug, and bed bug bites. Approximately 87% of FS patients reported being bitten by black flies; 60%, by kissing bugs; and 63%, by bed bugs. A much smaller proportion of controls reported bites from kissing bugs and bed bugs (29%–34%). Although contact with rodents was higher in FS patients (13.3%) than in controls (0.9% for community controls and 2.2% for family controls), the prevalence of exposure was low.

Table 4. Animals and insects in or around the home environment of study participants

Type of animal or insect	Cases (N = 30)	Community Controls (N = 108)	Family Controls (N = 90)
Dogs			
Yes	27 (90%)	89 (82.4%)	74 (82.2%)
No	3 (10%)	19 (17.6%)	16 (17.8%)
Cats			
Yes	18 (60.0%)	53 (49.1%)	46 (51.1%)
No	12 (40.0%)	55 (50.9%)	44 (48.9%)
Rats			
Yes	4 (13.3%)	16 (14.8%)	12 (13.3%)
No	26 (86.7%)	92 (85.2%)	78 (86.7%)
Chickens			
Yes	29 (96.7%)	103 (95.4%)	79 (87.8%)
No	1 (3.3%)	5 (4.6%)	11 (12.2%)
Cockroaches			
Yes	14 (46.7%)	49 (45.4%)	42 (46.7%)
No	16 (53.3%)	59 (54.6%)	48 (53.3%)
Bedbugs			
Yes	20 (66.7%)	53 (49.1%)	48 (42.2%)
No	10 (33.3%)	55 (50.9%)	52 (57.8%)
Kissing bugs			
Yes	26 (53.3%)	33 (30.6%)	41 (34.4%)
No	14 (46.7%)	75 (69.4%)	59 (65.6%)
Insect control in house as determined by interviewer			
Very good to good	0	9 (8.4%)	11 (12.2%)
Average	10 (34.5%)	35 (32.7%)	32 (35.6%)
Poor	10 (34.5%)	46 (43.0%)	42 (46.7%)
Very poor	9 (31.0%)	17 (15.9%)	5 (5.6%)
Missing	1	1	0

Table 5. Contact with insects and domestic animals reported by study participants

Insect bites	Cases (N = 30)	Community Controls (N = 108)	Family Controls (N = 90)
Black fly bites			
Yes	26 (86.7%)	87 (80.6%)	69 (76.7%)
No	4 (13.3%)	21 (19.4%)	21 (23.3%)
Kissing bug bites			
Yes	18 (60.0%)	31 (28.7%)	29 (32.2%)
No	12 (40.0%)	77 (71.3%)	61 (67.8%)
Bed bug bites			
Yes	19 (63.3%)	35 (32.4%)	31 (34.4%)
No	11 (36.7%)	73 (67.6%)	59 (65.6%)
Mosquito bites			
Yes	23 (76.7%)	77 (71.3%)	61 (67.8%)
No	7 (23.3%)	31 (28.7%)	29 (32.2%)
Tick bites			
Yes	1 (3.3%)	6 (5.6%)	8 (8.9%)
No	29 (96.7%)	102 (94.4%)	82 (91.1%)
Flea bites			
Yes	0 (0.0%)	0 (0.0%)	2 (2.2%)
No	30 (100%)	108 (100%)	88 (97.8%)
Exposure to rodents			
Yes	4 (13.3%)	1 (0.9%)	2 (2.2%)
No	26 (86.7%)	107 (99.1%)	88 (97.8%)
Exposure to cereal powders			
Yes	5 (16.7%)	23 (21.3%)	16 (17.8%)
No	25 (83.3%)	85 (78.7%)	74 (82.2%)
Exposure to cows			
Yes	2 (6.7%)	4 (3.7%)	6 (6.7%)
No	28 (93.3%)	104 (96.3%)	84 (93.3%)
Exposure to horses			
Yes	2 (6.7%)	0 (0.0%)	5 (5.6%)
No	28 (93.3%)	108 (100%)	85 (94.4%)
Exposure to dogs			
Yes	19 (63.3%)	82 (75.9%)	74 (82.2%)
No	11 (36.7%)	26 (24.1%)	16 (17.8%)
Exposure to cats			
Yes	10 (33.3%)	36 (33.3%)	26 (28.9%)
No	20 (66.7%)	72 (66.7%)	64 (71.1%)
Exposure to pigs			
Yes	4 (13.3%)	16 (14.8%)	18 (20.0%)
No	26 (86.6%)	92 (85.2%)	72 (80.0%)
Exposure to chickens			
Yes	22 (73.3%)	92 (85.2%)	75 (83.3%)
No	8 (26.7%)	16 (14.8%)	15 (16.7%)

These studies suggest that individuals living in Limao Verde might be at risk of developing FS if they live in rustic houses with thatched roofs, adobe walls, and poorly fitted or absent doors. Further, the chances of developing FS might be increased if these individuals are exposed to hematophagous bites (from bed bugs or kissing bugs). It is interesting to note that the housing of FS patients is similar to that inhabited by patients with Chagas disease, in which a kissing bug (*Reduviid* sp.) is involved in the transmission of disease (Despommier DD, Gwadz RW, Hotez PJ; Prata, 1999). Chagas disease is unknown in Limao Verde, however. Of interest, though, is that a previous investigation showed that the predominant species of black fly in Limao Verde is *Simulium nigrimanum* (Eaton et al, 1998), which in these regions of Brazil is not a vector of human diseases such as onchocerciasis.

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