Am. J. Trop. Med. Hyg., 87(4), 2012, pp. 675–680
doi:10.4269/ajtmh.2012.12-0206

Profile of *Trypanosoma cruzi* Reactivity in a Population at High Risk for Endemic Pemphigus Foliaceus (Fogo Selvagem)

Joaquim X. Sousa Jr., Luis A. Diaz, Donald P. Eaton, Günter Hans-Filho, Elder Lanzani de Freitas, Livia Delgado, Ligia Maria F. Ichimura, Flávia Cristaldi, Renata Orlandi, Norival Kesper Jr., Eufrosina S. Umezawa, Evandro A. Rivitti, Valeria Aoki,* and The Cooperative Group on Fogo Selvagem Research

Department of Dermatology, University of São Paulo Medical School, São Paulo, Brazil; Department of Dermatology, University of North Carolina at Chapel Hill, North Carolina; Wildlife Conservation Society, Campo Grande, MS, Brazil; Federal University of Mato Grosso do Sul, Brazil; Instituto de Medicina Tropical, University of São Paulo Medical School, São Paulo, Brazil

Abstract. Fogo Selvagem (FS) is an autoimmune bullous disease with pathogenic IgG autoantibodies recognizing desmoglein 1 (Dsg1), a desmosomal glycoprotein. In certain settlements of Brazil, a high prevalence of FS (3%) is reported, suggesting environmental factors as triggers of the autoimmune response. Healthy individuals from endemic areas recognize nonpathogenic epitopes of Dsg1, and exposure to hematophagous insects is a risk factor for FS. Fogo selvagem and Chagas disease share some geographic sites, and anti-Dsg1 has been detected in Chagas patients. Indeterminate Chagas disease was identified in a Brazilian Amerindian population of high risk for FS. In counterpart, none of the FS patients living in the same geographic region showed reactivity against *Trypanosoma cruzi.* The profile of anti-Dsg1 antibodies showed positive results in 15 of 40 FS sera and in 33 of 150 sera from healthy individuals from endemic FS sites, and no cross-reactivity between Chagas disease and FS was observed.

INTRODUCTION

Pemphigus foliaceus (PF) is an autoimmune bullous dermatosis driven by immunoglobulin G (IgG) autoantibodies that recognize glycoproteins involved in epidermal adhesion. The clinical expression of the autoimmune process is blister formation, consequent to epidermal detachment (acantholysis). These autoantibodies bind to the extracellular domains of desmoglein 1 (Dsg1), a cadherin located in the desmosomal core of the keratinocyte surface.[1–4]

There are two main forms of PF: the classic one, with universal distribution, and the endemic form, also known as Fogo Selvagem (FS), prevalent in certain regions of Brazil and other Latin American countries.[1,5] Main differences between the classic and the endemic presentation include peculiar epidemiological features, which are unique to FS, such as the presence of familial cases, involvement of children and young adults, and specific endemic settlements.[6]

The peak of FS in Brazil occurred in the first half of the 20th century. Aranha-Campos reported 604 cases through 1880 to 1940, where 26.5% were blood related[7]: a decline of the disease, concurrent with the development of the settlements has been observed. New foci in the Midwestern Brazilian States (Goiás, Mato Grosso, Mato Grosso do Sul) reported yearly incidences varying from 0.09 cases/10,000 inhabitants to 0.83 cases/10,000 inhabitants.[8] Frequency of 30.7 FS cases/year through 1990 to 1999 in the State of Mato Grosso do Sul has been detected.[9] Endemic sites of PF were also found in other countries such as Colombia, Venezuela, Paraguay, and Peru.[10–14]

Fogo Selvagem has a complex pathogenesis, which includes genetic, immunological, and environmental factors. A Brazilian Amerindian Terena reservation, located at Limao Verde, Aquidauana, State of Mato Grosso do Sul (MS), with a high prevalence of FS (3%), has been closely followed up, once its main features includes a geographic, limited distribution of FS cases, that exhibit familial and temporal clustering.[5,15–17]

The immune response in FS is characterized by pathogenic IgG4 auto-antibodies that are driven to the extracellular 1 and 2 domains of Dsg1 (EC1-2).[18] Interestingly, 55% of healthy individuals living in endemic FS areas generate anti-Dsg1 antibodies that recognize the extracellular 5 domain of Dsg1 (EC-5), a nonpathogenic epitope of the molecule. In those genetic predisposed individuals, intra-molecular spreading may occur, leading to an EC1-2-oriented IgG4 response, and therefore precipitating FS onset.[6,18] There is also evidence of other immunoglobulin classes in FS pathogenesis: circulating IgM autoantibodies directed against Dsg1 are found in FS patients and in healthy individuals living in endemic areas, indicating a role as serological markers for the disease[19]; moreover, an IgE-based immune response to Dsg1 was detected in the sera of 81% of FS patients.[20] These findings lead to the hypothesis of continuous exposure to an environmental antigen that may share epitopes to Dsg1, and become a strong stimulus to nonpathogenic anti-Dsg1 IgM and IgG production in areas at high risk for FS.[20]

The genetic influence on FS is characterized by a positive association with the human leukocyte antigen alleles HLA-DRB1-0404, -1402 or -1406, with a relative risk of 14. A sequence of eight amino acids (LLEQRRAA) at the positions 67–74 in the third hypervariable domain of the DRB1 gene is shared by these alleles, conferring susceptibility to the disease.[21,22]

In genetically predisposed individuals, there may be triggers that initiate the immune response in FS through an antigen mimicry process.[16] It is hypothesized that a break of immune tolerance follows exposure to some environmental factor(s) that include hematophagous insect bites, as reported elsewhere.[16,23,24] The potential role of black fly triggering the autoimmune response in FS is supported by two main studies: exposure to simuliid bites as a risk factor for FS (4.7 odds ratio),[23] and the predominance of a certain black fly species (*Simulium nigrimanum*) in endemic areas, when

* Address correspondence to Valeria Aoki, Av. Dr. Enéas de Carvalho Aguiar 255, Sala 3016, Departamento de Dermatologia-Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, CEP 05403-002. E-mail: valeria.aoki@gmail.com

compared with non-endemic areas of the Brazilian coast.[24] Additional data on the environment triggers is suggested by Aoki and others,[16] who reported a high frequency of black fly (87%), kissing bugs (67%), and bed bugs (60%) bites in FS patients, and showed that precarious living conditions represented a significant risk factor for FS. The sialotranscriptome of *S. nigrimanum*, the most common black fly species in endemic FS areas has been isolated, comprising over 70 distinct genes within over 30 protein families, offers an infinite source for testing pemphigus patients.[25]

Some geographic areas of Brazilian vector-mediated tropical diseases, such as cutaneous leishmaniasis and Chagas disease, coincide with those endemic areas of FS. A previous work from our group evaluated the prevalence of anti-desmoglein 1 antibody in patients with cutaneous leishmaniasis, onchocerciasis, and Chagas disease, detecting a high prevalence of circulating autoantibodies directed against the nonpathogenic extracellular domain 5 of Dsg1 in Chagas disease (58%), leishmaniasis (43%), and onchocerciasis (81%).[17]

Chagas disease is one of the major causes of cardiac chronic disease in Latin America, and is endemic in many regions of Brazil. However, in the State of Mato Grosso do Sul (MS), it is not considered an endemic disease[26] because of the measures adopted for the vector's control. *Triatoma infestans*, the main vector for Chagas disease in Brazil, has a low density in MS. *Triatoma infestans* was found among different regions in MS in the end of the 20th century (1980–2000), but it has been seldom detected in the last decade. On the other hand, different Triatominae species such as *Triatoma sordida, Panstrongylus geniculatus*, and *Rhodnius neglectus* with infection rates by *Trypanosoma cruzi* varying from 0.1% to 3.2% have been reported in this geographic region.[26]

Information about the reactivity against *T. cruzi* of individuals from endemic areas of FS in the State of Mato Grosso do Sul is scarce. Therefore, this study aimed to characterize the immune response to *T. cruzi* in a population at high risk for FS.

MATERIALS AND METHODS

Geographic location of the endemic site. Limao Verde (LV) reservation is located 25 km Northeast of Aquidauana, and 160 km West of Campo Grande, the capital of the State of Mato Grosso do Sul (55°41′07″W, 20°19′00″S), as described elsewhere.[27] It comprises a total of 1,712 hectares, with two well-defined geographical regions, LV and Corrego Seco. In September 2011, 1,349 people among 295 families inhabited LV reservation. The overall prevalence of FS in this endemic site was 3% in previous reports.[27] Most of the houses have dirt floors, adobe walls, thatched roofs, poorly fitted or nonexistent doors, and no indoor plumbing or electricity. Poor toilet facilities are a common feature. The majority of individuals sleep on a raised platform covered by a variety of bedding material.[16]

Study design. We included 40 FS patients from the LV Terena Reservation, MS, a FS focus where a clinical and immunological surveillance has been started since 1993[17] and 150 healthy individuals (selected at random from a total population of 1,349 inhabitants of LV) from September 2008 to September 2011. Blood samples were obtained by venipuncture and frozen at −70°C. All FS patients fulfilled clinical, histological, and immunofluorescence criteria for PF.

All participants were informed about the study and signed an informed consent, approved by the Ethics Committee (CAPPesq) from our institution.

Serologic assays. The collected samples of the selected individuals (FS patients and controls from LV) were analyzed for serological response against *T. cruzi* epimastigotes (Biomérieux, Marcy l'Etoile, France), using enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence (IIF) with IgM and IgG (Biocientífica SA, Buenos Aires, Argentina) following standard procedures, according to manufacturers' instructions as described elsewhere.[28,29]

Immunoblotting with trypomastigote excreted-secreted antigens (TESA blot), an assay used as a confirmatory test for Chagas disease was performed in the five IIF positive sera to rule out cross-reactivity with leishmaniasis, and is briefly described as follows[30]:

The TESA from the Y strain of *T. cruzi* were obtained as previously described.[30] Briefly, the supernatants of LLC-MK2 cell cultures (in serum-free medium or with 2% fetal calf serum) infected with *T. cruzi* were collected when the concentration of trypomastigotes reached about 10–20 × 10^6/mL. After being centrifuged at 1800 × *g* for 15 min at 4°C, the supernatant containing TESA was then resubmitted to a second centrifugation (7000 × *g* for 5 min at 4°C) and used directly without any further treatment or stored at −80°C in small aliquots.

Proteins from TESA were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose sheets, and blocked with phosphate buffered saline (PBS) containing 5% fat-free milk for 1 h at room temperature. Membrane strips (5 mm) were incubated with human sera (1:200) diluted in PBS with 1% milk for 2 h or overnight at room temperature, washed, and the bound antibodies were detected with horseradish peroxidase (HRP)-labeled anti-human IgG (Sigma, St. Louis, MO), diluted (1:4000). The color of detected bands was developed by addition of 0.1% hydrogen peroxide and 4-chloro-1-naphthol. Samples were considered positive when a large 150–160 kDa band and/or five bands between 130 and 200 kDa were observed.

The serological profile of all sera (total IgG) against a recombinant form of human Dsg1, containing the entire extracellular domain and a C-terminal His-tag, described elsewhere, was tested by ELISA.[6,18,31] Briefly, the ectodomain of Dsg-1 with a carboxy-terminal His-tag were produced in High Five insect cells by infection with the recombinant baculovirus stock of Dsg-1 (UNC Immunodermatology Laboratory, Department of Dermatology, University of North Carolina at Chapel Hill, NC). To generate the deglycosylated Dsg1, tunicamycin (Sigma) was added to the culture medium (0.5 μg/mL) at the time of infection. The tunicamycin-treated (deglycosylated) and untreated (glycosylated) recombinant Dsg1 was purified by nickel affinity chromatography and used for ELISA assay. The ELISA plates were coated with 200 ng/well of purified Dsg1 at 4°C overnight. After washing with Tris-buffered saline containing 3.7 mM Ca^{++} and 0.05% Tween-20 (TBS/Ca^{++}/T-20), the plate was blocked with 1% bovine serum albumin (BSA) in TBS/Ca^{++}/T-20 at room temperature for 1h. The plate was then incubated with duplicate 1:100 dilutions of serum samples for 1 h at room temperature. Following wash, the plate was incubated with a 1:1000 dilution of HRP-labeled mouse anti-human IgG or with 1:2000 dilution of HRP-conjugated mouse anti-human IgG (Zymed, San Francisco, CA). Results were expressed as index

value units, and a cut-off value of 20 arbitrary units was used to separate positive from negative sera; values > 20 were considered positive.

RESULTS

Age and gender distribution. *FS patients.* There was a slight predominance of male patients (22 male: 18 female) and the median age was 32 years (ages ranging from 12 to 76 years). Close familial clustering was present in 34 of 40 (85%) of the patients, mostly parents/children or siblings. As for ethnic distribution, Terena Indians comprised the majority (32 of 40), and eight individuals were mestizos of Terena origin.

Non-FS individuals. In this clustered group of 150 individuals from LV, with a female predominance (89 female: 61 male), ages varied from 4 to 92 years, median age of 22.5 years; ethnic distribution revealed that 57% were Terena Indians and 43% mestizos.

Chagas disease assays. *ELISA.* The ELISA assays using *T. cruzi* epimastigotes for Chagas disease detected a negative response in 39 but one FS patient (cutoff 0.303, median 0.304) (Table 1).

The ELISA assays using *T. cruzi* epimastigotes for Chagas disease detected 5 of 150 non-FS patients from LV (cutoff 0.303, median 0.304) (Table 2).

IIF. None of the 40 FS sera showed reactivity against *T. cruzi* epimastigotes by IIF. The IgG antibodies that recognized *T. cruzi* epimastigotes were detected in 5 of 150 non-FS sera, titers varying from IIF 1:320 to > 1:640. Three of five individuals shared the same house, and were blood-related to FS patients. One of five individuals showed both IgM and IgG antibodies directed to *T. cruzi*.

TESA-blot. Five non-FS individuals confirmed indeterminate Chagas disease by positive results using immunoblotting with trypomastigote excreted-secreted antigens (Figure 1A and B).

Recombinant desmoglein 1 ELISA assay (total IgG). *FS patients.* Fifteen of 40 FS sera showed positive results by rDsg1 (Table 1), index values varying from 23 to 422 (mean: 133). The FS sera with negative rDsg1 results by ELISA corresponded to those in FS remission.

Non-FS individuals. Thirty-three healthy individuals out of 150 (22%) recognized rDsg1 by ELISA (Table 2), index values ranging from 20 to 227 (mean: 86). It is noteworthy to report that none of the non-FS individuals that recognized *T. cruzi* antigens showed positive ELISAs for rDsg1.

DISCUSSION

Endemic PF or FS represents a unique model of an autoimmune condition that may be triggered by environmental factors in genetic-prone individuals. The antigenic target is the extracellular portion of desmoglein 1, an adhesion molecule of the cadherin superfamily, which is recognized by pathogenic IgG, especially of the IgG4 isotype.[6,22]

The recognition of Dsg 1 epitopes is not restricted to patients with the disease, as previously reported.[8] In this study, we detected 22% of reactivity against Dsg1 in non-FS individuals, indicating a possible environmental stimulus in the development of autoantibody formation. Some findings concerning the nonpathogenic response towards Dsg1 are relevant to reinforce the environmental hypothesis in FS, as follows: the lower prevalence of the IgG anti-Dsg1 response in normal subjects that live far away from the endemic sites (13%),[32] the predominance of a certain black fly species, *Simulium nigrimanum,* in FS regions,[24] an enhanced IgM or IgE anti-Dsg1 immune response in FS,[19,20] and finally, the epidemiological data emphasizing the relevance of housing conditions and exposure to hematophagous insects, such as bedbugs and kissing bugs of patients with the disease.[16]

Individuals with parasitic diseases that are vector-mediated, such as onchocerciasis, cutaneous leishmaniasis, and Chagas

TABLE 1
Chagas disease serology and autoantibodies anti-desmoglein 1 in patients with Fogo Selvagem using ELISA*

	Chagas disease			
Fogo Selvagem (rDsg1)	Positive	Negative	Indetermined	Total
Positive	0	15	0	15
Negative	0	24	1	25
Total	0	39	1	40

* ELISA = enzyme-linked immunosorbent assay; rDsg1 = recombinant Desmoglein 1.

TABLE 2
Chagas disease profile and autoantibodies anti-desmoglein 1 tested by ELISA in non-Fogo Selvagem individuals from Limao Verde, MS

	Chagas disease	
Non-FS individuals (rDsg1)	Positive	Negative
Positive	0	33
Negative	5	112
Total	5	145

FS = Fogo Selvagem; rDsg1 = recombinant Desmoglein 1.

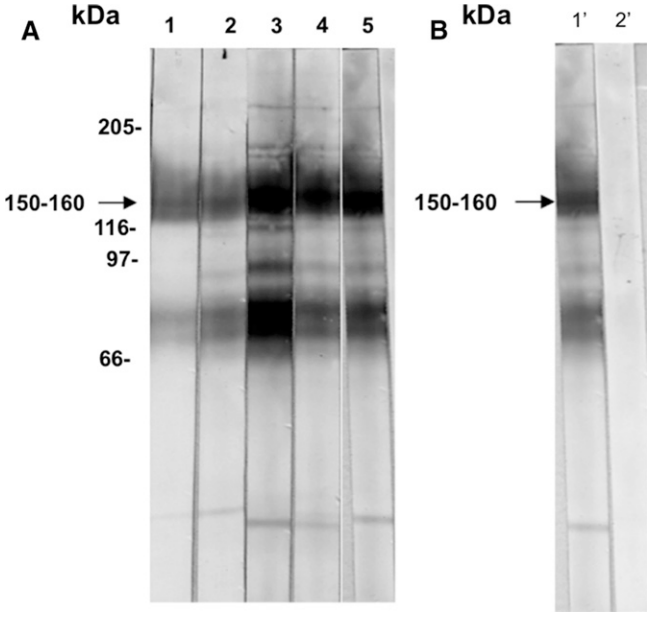

FIGURE 1. (**A**) Immunoblotting with trypomastigote excreted-secreted antigens (TESA blot) polypeptides recognized by IgG (lanes 1 to 5) from non-FS patients' sera from Limao Verde (LV). (**B**) Lanes 1′ and 2′, positive and negative controls, respectively. Patients in lanes 1, 2, 3, 4, and 5 were classified as having indeterminate forms of Chagas disease, recognizing a 150- to 160-kDa band (chronic-phase antigen). Molecular mass markers are on the left. The molecular mass standards used were 205 kDa (rabbit muscle myosin); 116 kDa (*Escherichia coli* galactosidase); 97 kDa (rabbit muscle phosphorylase b); 66 kDa (bovine serum albumin).

disease, often possess circulating nonpathogenic anti-Dsg1 autoantibodies.[17] In the pre-clinical phase of FS, there is an antigen-driven selection of anti-Dsg1 B cells,[33] but the real source of this antigen remains to be determined.

Chagas disease is a major public health problem in many Latin America countries, but there are differences about its epidemiology in Amerindian populations living in these geographic sites.[34] In native South American populations located in highlands (i.e., Bolivia, Argentina, Chile), records of Chagas disease date since the pre-Columbian era.[34] Meanwhile, there is no report of the disease among lowland Amerindians or of *Triatoma* sp. living in their traditional houses.[35]

Among the native Brazilian population, there is no evidence of *Trypanosoma* sp. in Xingu, Asurini Indians, Karitiana and Surui Indians.[35] Coimbra[36] performed a serological survey with the Xavante Indians from Mato Grosso, and found negative results in all 168 individuals tested for Chagas disease.

It has been hypothesized that Chagas disease is endemic in natives from the highlands because of features related to early domiciliation of triatomines and maintenance of the domestic cycle of *T. cruzi*.[36,37] On the other hand, Amerindians in the lowland used to live in small settlements, with high village mobility and absence of domestic animals, similar to the conditions observed among native populations studied in Brazil.[36]

The Terena population of LV reservation shows closer features with those populations of the highland Amerindians, i.e., animal domestication, raising indoor chickens, and low mobility. These conditions, when associated with the house with thatched roofs or adobe walls (Figure 2) provide an adequate environment to the domiciliation process of many species of triatomines. Our entomologist (DPE) performed an entomologic survey in LV, and detected four predominant Reduviidae species: *Triatoma matogrossensis*, *Triatoma sordida*, *Rhodnius prolixus*, and *Panstrongylus geniculatus*. Rates of natural infection with *T. cruzi* have been not yet recorded for those species (Eaton and others, unpublished data). These data are corroborated by previous performed studies on the profile of Reduviidae in a domestic environment in the State of Mato Grosso do Sul.[26]

Although there were reports of the presence of triatomine inside the houses, and frequent exposure to kissing bugs in previous studies performed in the LV reservation, no Chagas disease has been so far detected in this endemic FS site, which has been followed up since 1993.[15,16] To date, we did not find cross-reactivity with desmoglein 1 among *T. cruzi* positive sera in our samples, suggesting no relationship between *T. cruzi* and FS. In Brazil, there is a single report on IgG reactivity (38%) against trypomastigote forms of *T. cruzi* in eight sera from PF patients by IIF. However, there was no further information about those PF patients, and relevant data such as geographic location and demographic characteristics of these individuals were not available.[38]

FIGURE 2. Typical house in Limao Verde - Terena Reservation, MS, Brazil.

The mechanisms involved in anti-Dsg1 autoantibodies formation in patients with Chagas disease remain unknown. One of the possibilities includes compounds of hematophagous insect saliva inducing an immune response against Dsg1.[17] It is interesting to note that antibodies of the IgG4 subclass directed against *Triatoma infestans* salivary gland proteins are produced by individuals living in triatomine-infested areas.[39] Further studies are necessary to improve our understanding of such mechanisms; Assumpção and others[40] recently described sialotranscriptome of *T. matogrossensis* from high-risk areas for FS, which may facilitate the identification of antigens with potential role for triggering FS, as well as development of biomarkers for low-level infestation of triatomines.

Conventional methods such as ELISA and IIF have been established for the serologic diagnosis of Chagas disease. In previous studies, ELISA sensitivity varied from 97.7% to 100%, and specificity, 93.3% to 100%; IIF showed sensitivity from 72% to 100% and specificity from 96% to 100%. Accuracy of both assays displays the best results in indeterminate and chronic stages, although there is frequent cross-reactivity, especially with leishmaniasis.[41,42] Fortunately, TESA is a diagnostic method for Chagas disease, with 100% specificity, showing no cross-reaction for the 130- to 200-kDa antigen (acute-phase antigens) or the 150- to 160-kDa antigens (chronic-phase antigens).[30] In our samples, TESA blot (Figure 1) showed bands that correspond to chronic-phase antigens, confirming the indeterminate stage for all five positive non-FS individuals from endemic areas of FS, previously tested by ELISA and IIF.

The description of reactivity against desmogleins 1 and 3 has been reported in patients from Tunisia, with visceral leishmaniasis (22%) and hidatidosis (40%). In counterpart, no significant difference was found in PF patients and controls concerning the immune response against the above parasitic agents.[43] Similarly, Brazilian patients with mucocutaneous leishmaniasis did show reactivity against Dsg1 in 43% of the cases; however, all FS patients from LV, except one, developed mucocutaneous leishmaniasis when tested by indirect immunofluorescence and ELISA (Diaz and others, unpublished data).

Pathophysiology of Chagas disease is complex and has been related to an autoimmune process. Immune response in *T. cruzi* chronic infection has features of delayed-type hypersensitivity with predominance of $CD8^+$ over $CD4^+$ T-cell subsets. Although cell-mediated immunity plays a central role in that process, humoral immunity may have participation through IgG directed against self-antigens such as neurons, sciatic nerve homogenates, and small nuclear ribonucleoproteins. Moreover, complement membrane attack complexes were identified in cardiac myocytes from Chagas disease patients.[44]

Similar to FS, molecular mimicry appears as an important phenomenon in autoimmunity of Chagas disease. *Trypanosoma cruzi* antigens such as B13 protein, microsomal fraction,

sulfated glycolipids, and ribosomal protein have been described as molecules that may induce cross-reactivity with self-antigens found in human heart muscle (cardiac myosin, human ribosomal protein) or nervous tissue. Yet, anti-neuron autoantibodies found in Chagas disease may be linked to autonomic nervous system dysfunction in those patients. Moreover, T-cell clones sensitized to B13-protein in chronic Chagas disease with cardiomyopathy have been identified, showing multiple cross-reactive epitopes between *T. cruzi* B13 protein and human cardiac myosin heavy chain.[44]

Our study revealed the occurrence of indeterminate Chagas disease in an Amerindian Terena population at high risk for FS in Brazil. Despite the absence of coexistence of the two conditions, clinical, epidemiological, and immune surveillance for FS and Chagas disease in this endemic area is mandatory, once both conditions share the same environmental *milieu*.

Received March 31, 2012. Accepted for publication June 22, 2012.

Financial support: This work was partially supported by a National Institute of Health grant-NIAMSD 5 R01 AR032599-29 (LAD).

Disclaimer: The authors declare no conflicts of interest.

Authors' addresses: Joaquim X. Sousa Jr., Elder Lanzani de Freitas, Livia Delgado, Ligia Maria F. Ichimura, Flávia Cristaldi, Renata Orlandi, Evandro A. Rivitti, and Valeria Aoki, Departamento de Dermatologia-Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, E-mails: jxsj@yahoo.com, elderlanzanifreitas@yahoo.com.br/, paralivi@hotmail.com, liichi01@hotmail.com, flaviacristaldi@bol.com.br, re_orlandi@hotmail.com/, evandro.rivitti@gmail.com, and valeria.aoki@gmail.com. Luis A. Diaz, Department of Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, NC, E-mail: ldiaz@med.unc.edu. Donald P. Eaton, Wildlife Conservation Society, Campo Grande, MS, Brazil, E-mail: ksadeaton@yahoo.com. Günter Hans-Filho, Department of Dermatology, Federal University of Mato Grosso do Sul, Campo Grande, Brazil, E-mail: ghansfilho@hotmail.com. Norival Kesper Jr. and Eufrosina S. Umezawa, Instituto de Medicina Tropical, University of São Paulo Medical School, São Paulo, Brazil, E-mails: nkesper@usp.br and eumezawa@usp.br.

REFERENCES

1. Sampaio SA, Rivitti EA, Aoki V, Diaz LA, 1994. Brazilian pemphigus foliaceus, endemic pemphigus foliaceus, or fogo selvagem (wild fire). *Dermatol Clin 12:* 765–776.
2. Aoki V, Huang MH, Perigo AM, Fukumori LM, Maruta CW, Santi CG, Oliveira ZN, Rivitti E, 2004. Endemic pemphigus foliaceus (fogo selvagem) and pemphigus vulgaris: immunoglobulin G heterogeneity detected by indirect immunofluorescence. *Rev Hosp Clin Fac Med Sao Paulo 59:* 251–256.
3. Amagai M, 1995. Adhesion molecules. I: keratinocyte-keratinocyte interactions; cadherins and pemphigus. *J Invest Dermatol 104:* 146–152.
4. Nilles LA, Parry DA, Powers EE, Angst BD, Wagner RM, Green KJ, 1991. Structural analysis and expression of human desmoglein: a cadherin-like component of the desmosome. *J Cell Sci 99:* 809–821.
5. Culton DA, Qian Y, Li N, Rubenstein D, Aoki V, Filhio GH, Rivitti EA, Diaz LA, 2008. Advances in pemphigus and its endemic pemphigus foliaceus (Fogo Selvagem) phenotype: a paradigm of human autoimmunity. *J Autoimmun 31:* 311–324.
6. Warren SJ, Lin MS, Giudice GJ, Hoffmann RG, Hans-Filho G, Aoki V, Rivitti EA, Santos V, Diaz LA, 2000. The prevalence of antibodies against desmoglein 1 in endemic pemphigus foliaceus in Brazil. Cooperative Group on Fogo Selvagem Research. *N Engl J Med 343:* 23–30.
7. Aranha-Campos J, 1942. *Pemphigus foliaceus (Fogo Selvagem): Clinical and Epidemiological Aspects*. Sao Paulo, Brazil: Comp Melhoramentos.
8. Chiossi MP, Roselino AM, 2001. Endemic pemphigus foliaceus ("Fogo selvagem"): a series from the northeastern region of the State of Sao Paulo, Brazil, 1973–1998. *Rev Inst Med Trop Sao Paulo 43:* 59–62.
9. Chagas AC, Ivo ML, Honer MR, Correa Filho R, 2005. Situation of endemic pemphigus foliaceus in Mato Grosso do Sul, Brazil, 1990–1999. *Rev Lat Am Enfermagem 13:* 274–276.
10. Robledo MA, Prada S, Jaramillo D, Leon W, 1988. South American pemphigus foliaceus: study of an epidemic in El Bagre and Nechi, Colombia 1982 to 1986. *Br J Dermatol 118:* 737–744.
11. Gonzalez F, Saenz AM, Cirocco A, Tacaronte IM, Fajardo JE, Calebotta A, 2006. Endemic pemphigus foliaceus in Venezuela: report of two children. *Pediatr Dermatol 23:* 132–135.
12. Aldama A, Correa J, Rivelli V, Mendoza G, 2000. Tipos y variantes de pénfigo en el Hospital Nacional de Paraguay. Revisión de 70 casos. *Med Cut ILA:* 242–247.
13. Aldama A, Gorostiaga G, Rivelli V, Mendoza G, Domínguez L, 2006. Pénfigo foliceo endémico en menores de 20 años en Paraguay. *Dermatol Pediatr Lat 4:* 111–114.
14. Ortega Loayza AG, Ramos W, Elgart G, Bouman P, Jimenez G, Avila J, Rojas I, Vilcarromero M, Hurtado J, Lindo G, Galarza C, 2006. Antibodies against desmoglein 1 in healthy subjects in endemic and nonendemic areas of pemphigus foliaceus (fogo selvagem) in Peru. *Int J Dermatol 45:* 538–542.
15. Hans-Filho G, Aoki V, Rivitti E, Eaton DP, Lin MS, Diaz LA, 1999. Endemic pemphigus foliaceus (fogo selvagem)–1998. The Cooperative Group on Fogo Selvagem Research. *Clin Dermatol 17:* 225–235, discussion 105–106.
16. Aoki V, Millikan RC, Rivitti EA, Hans-Filho G, Eaton DP, Warren SJ, Li N, Hilario-Vargas J, Hoffmann RG, Diaz LA, 2004. Environmental risk factors in endemic pemphigus foliaceus (fogo selvagem). *J Investig Dermatol Symp Proc 9:* 34–40.
17. Diaz LA, Arteaga LA, Hilario-Vargas J, Valenzuela JG, Li N, Warren S, Aoki V, Hans-Filho G, Eaton D, dos Santos V, Nutman TB, de Mayolo AA, Qaqish BF, Sampaio SA, Rivitti EA, 2004. Anti-desmoglein-1 antibodies in onchocerciasis, leishmaniasis and Chagas disease suggest a possible etiological link to Fogo selvagem. *J Invest Dermatol 123:* 1045–1051.
18. Li N, Aoki V, Hans-Filho G, Rivitti EA, Diaz LA, 2003. The role of intramolecular epitope spreading in the pathogenesis of endemic pemphigus foliaceus (fogo selvagem). *J Exp Med 197:* 1501–1510.
19. Diaz LA, Prisayanh PS, Dasher DA, Li N, Evangelista F, Aoki V, Hans-Filho G, dos Santos V, Qaqish BF, Rivitti EA, 2008. The IgM anti-desmoglein 1 response distinguishes Brazilian pemphigus foliaceus (fogo selvagem) from other forms of pemphigus. *J Invest Dermatol 128:* 667–675.
20. Qian Y, Prisayanh P, Andraca E, Qaqish BF, Aoki V, Hans-Filho G, Rivitti EA, Diaz LA, 2011. IgE, IgM, and IgG4 anti-desmoglein 1 autoantibody profile in endemic pemphigus foliaceus (fogo selvagem). *J Invest Dermatol 131:* 985–987.
21. Moraes ME, Fernandez-Vina M, Lazaro A, Diaz LA, Filho GH, Friedman H, Rivitti E, Aoki V, Stastny P, Moraes JR, 1997. An epitope in the third hypervariable region of the DRB1 gene is involved in the susceptibility to endemic pemphigus foliaceus (fogo selvagem) in three different Brazilian populations. *Tissue Antigens 49:* 35–40.
22. Aoki V, Sousa JX Jr, Diaz LA, 2011. Pathogenesis of endemic pemphigus foliaceus. *Dermatol Clin 29:* 413–418.
23. Lombardi C, Borges PC, Chaul A, Sampaio SA, Rivitti EA, Friedman H, Martins CR, Sanches Junior JA, Cunha PR, Hoffmann RG, et al., 1992. Environmental risk factors in endemic pemphigus foliaceus (Fogo selvagem). The Cooperative Group on Fogo Selvagem Research. *J Invest Dermatol 98:* 847–850.
24. Eaton DP, Diaz LA, Hans-Filho G, Santos VD, Aoki V, Friedman H, Rivitti EA, Sampaio SA, Gottlieb MS, Giudice GJ, Lopez A, Cupp EW, 1998. Comparison of black fly species (Diptera: Simuliidae) on an Amerindian reservation with a high prevalence of fogo selvagem to neighboring disease-free sites in the State of Mato Grosso do Sul, Brazil. The Cooperative Group on Fogo Selvagem Research. *J Med Entomol 35:* 120–131.
25. Ribeiro JM, Valenzuela JG, Pham VM, Kleeman L, Barbian KD, Favreau AJ, Eaton DP, Aoki V, Hans-Filho G, Rivitti EA, Diaz

LA, 2010. An insight into the sialotranscriptome of *Simulium nigrimanum*, a black fly associated with fogo selvagem in South America. *Am J Trop Med Hyg 82:* 1060–1075.
26. Almeida PS, Ceretti Junior W, Obara MT, Santos HR, Barata JM, Faccenda O, 2008. Survey of Triatominae (Hemiptera: Reduviidae) fauna in domestic environments and natural infection by Trypanosomatidae in the State of Mato Grosso do Sul. *Rev Soc Bras Med Trop 41:* 374–380.
27. Hans-Filho G, dos Santos V, Katayama JH, Aoki V, Rivitti EA, Sampaio SA, Friedman H, Moraes JR, Moraes ME, Eaton DP, Lopez AL, Hoffman RG, Fairley JA, Giudice GJ, Diaz LA, 1996. An active focus of high prevalence of fogo selvagem on an Amerindian reservation in Brazil. Cooperative Group on Fogo Selvagem Research. *J Invest Dermatol 107:* 68–75.
28. Ross A, Novoa-Montero D, 1993. Comparability and reliability of ELISA, immunofluorescence, and indirect hemagglutination assays for *Trypanosoma cruzi* and *Trypanosoma rangeli*. *J Infect Dis 168:* 1581–1584.
29. Carvalho MR, Krieger MA, Almeida E, Oelemann W, Shikanai-Yassuda MA, Ferreira AW, Pereira JB, Saez-Alquezar A, Dorlhiac-Llacer PE, Chamone DF, et al., 1993. Chagas' disease diagnosis: evaluation of several tests in blood bank screening. *Transfusion 33:* 830–834.
30. Umezawa ES, Nascimento MS, Kesper N Jr, Coura JR, Borges-Pereira J, Junqueira AC, Camargo ME, 1996. Immunoblot assay using excreted-secreted antigens of *Trypanosoma cruzi* in serodiagnosis of congenital, acute, and chronic Chagas' disease. *J Clin Microbiol 34:* 2143–2147.
31. Warren SJ, Arteaga LA, Rivitti EA, Aoki V, Hans-Filho G, Qaqish BF, Lin MS, Giudice GJ, Diaz LA, 2003. The role of subclass switching in the pathogenesis of endemic pemphigus foliaceus. *J Invest Dermatol 120:* 104–108.
32. Sousa JX Jr, Freitas E, Delgado L, Coelho T, Aoki V, 2009. Antidesmoglein 1 reactivity in a healthy Brazilian population at low risk for endemic pemphigus foliaceus (fogo selvagem). *J Am Acad Dermatol 60:* AB10.
33. Qian Y, Clarke SH, Aoki V, Hans-Filhio G, Rivitti EA, Diaz LA, 2009. Antigen selection of anti-DSG1 autoantibodies during and before the onset of endemic pemphigus foliaceus. *J Invest Dermatol 129:* 2823–2834.
34. Rothhammer F, Allison MJ, Nunez L, Standen V, Arriaza B, 1985. Chagas' disease in pre-Columbian South America. *Am J Phys Anthropol 68:* 495–498.
35. Coimbra CE, 1988. Human settlements, demographic pattern, and epidemiology in lowland Amazonia: the case of Chagas's disease. *Am Anthropol 90:* 82–97.
36. Coimbra Junior CE, Borges MM, Flowers NM, Santos RV, Piazza RF, 1992. Sero-epidemiological survey for Chagas' disease among the Xavante Indians of central Brazil. *Ann Trop Med Parasitol 86:* 567–568.
37. Lent H, Wygodzinsky PW, 1979. Revision of the *Triatominae* (*Hemiptera, Reduviidae*), and their significance as vectors of Chagas' disease. *Bull Am Mus Nat Hist 163:* 125–520.
38. Primavera KS, Umezawa ES, Peres BA, Camargo ME, Hoshino-Shimizu S, 1990. Chagas' disease: IgA, IgM and IgG antibodies to *T. cruzi* amastigote, trypomastigote and epimastigote antigens in acute and in different chronic forms of the disease. *Rev Inst Med Trop Sao Paulo 32:* 172–180.
39. Nascimento RJ, Santana JM, Lozzi SP, Araujo CN, Teixeira AR, 2001. Human IgG1 and IgG4: the main antibodies against *Triatoma infestans* (Hemiptera: Reduviidae) salivary gland proteins. *Am J Trop Med Hyg 65:* 219–226.
40. Assumpção TC, Eaton DP, Pham VM, Francischetti IM, Aoki V, Hans-Filho G, Rivitti EA, Valenzuela JG, Diaz LA, Ribeiro JM, 2012. An insight into the Sialotranscriptome of *Triatoma matogrossensis*, a kissing bug associated with fogo selvagem in South America. *Am J Trop Med Hyg 86:* 1005–1014.
41. Oelemann WM, Teixeira MD, Verissimo Da Costa GC, Borges-Pereira J, De Castro JA, Coura JR, Peralta JM, 1998. Evaluation of three commercial enzyme-linked immunosorbent assays for diagnosis of Chagas' disease. *J Clin Microbiol 36:* 2423–2427.
42. Malan AK, Avelar E, Litwin SE, Hill HR, Litwin CM, 2006. Serological diagnosis of *Trypanosoma cruzi*: evaluation of three enzyme immunoassays and an indirect immunofluorescent assay. *J Med Microbiol 55:* 171–178.
43. Kallel Sellami M, Zitouni M, Tombari W, Ben Ayed M, Abida O, Laadhar L, Mokni M, Fezza B, Turki H, Mokhtar I, Ben Osman A, Kamoun Mohamed R, Joly P, Tron F, Gilbert D, Masmoudi H, Makni S; Franco-Tunisian Group of Survey, Research on Tunisian Endemic Pemphigus, 2007. Anti-desmoglein-1 antibodies are prevalent in Tunisian patients with hydatidosis and leishmaniasis. *Br J Dermatol 156:* 591–593.
44. Cunha-Neto E, Bilate AM, Hyland KV, Fonseca SG, Kalil J, Engman DM, 2006. Induction of cardiac autoimmunity in Chagas heart disease: a case for molecular mimicry. *Autoimmunity 39:* 41–54.