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IgE Anti-LJM11 Sand Fly Salivary Antigen May Herald the Onset of Fogo Selvagem in Endemic Brazilian Regions

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TO THE EDITOR

Fogo selvagem (FS) is an endemic form of pemphigus foliaceus, which is prevalent in certain regions of Brazil (Diaz *et al.*, 1989). Genetic and environmental factors such as insect bites may contribute to the development of FS (Diaz *et al.*, 1989; Moraes *et al.*, 1997; Aoki *et al.*, 2004). Blister formation in FS is mediated by predominantly IgG4 anti-desmoglein 1 (Dsg1) antibodies (Rock *et al.*, 1989), which are predictors of FS (Qaqish *et al.*, 2009). Our recent studies demonstrate that FS IgG4 antibodies cross-react with LJM11, a salivary protein from sand fly *Lutzomyia longipalpis* (Qian *et al.*, 2012), suggesting that sand

fly bites may deliver the antigen(s) that have a role in driving the development of these IgG4 autoantibodies in FS.

The concomitant development of IgE and IgG4 has been well documented in allergy (Lichtenstein *et al.*, 1968; Muller, 2005). In addition, insect bites are known to induce IgE responses. IgE response in pemphigus vulgaris and FS has been described by Nagel *et al.* (2009) and us (Qian *et al.*, 2011, 2012). We have demonstrated that FS patients have significantly higher IgG4 and IgE against sand fly salivary gland antigens (SGLL) and monoclonal IgG4 autoantibodies derived from FS patients cross-react with LJM11, a major

immunogenic component from SGLL (Qian *et al.*, 2012). In this study, we seek to determine whether IgE antibodies to either autoantigen (Dsg1) and/or environmental antigen (LJM11) are present among individuals living in FS endemic regions. All serum samples from controls and patients in this investigation were obtained from individuals studied during the last 25 years and kept in a bank of sera at the University of North Carolina Dermatology Research Laboratories. These studies are approved by the institutional review boards from the University of North Carolina, Chapel Hill, and the University of Sao Paulo, Sao Paulo, Brazil. Randomly selected serum samples ($n = 30$) from FS patients, as well as serum samples of healthy control (HC) individuals from FS endemic region ($n = 32$), Limao Verde

Abbreviations: Dsg1, desmoglein 1; FS, fogo selvagem; HC, healthy control; LV, Limao Verde in Brazil; RLUs, relative light units; SGLL, salivary gland antigens from sand fly (*Lutzomyia longipalpis*)

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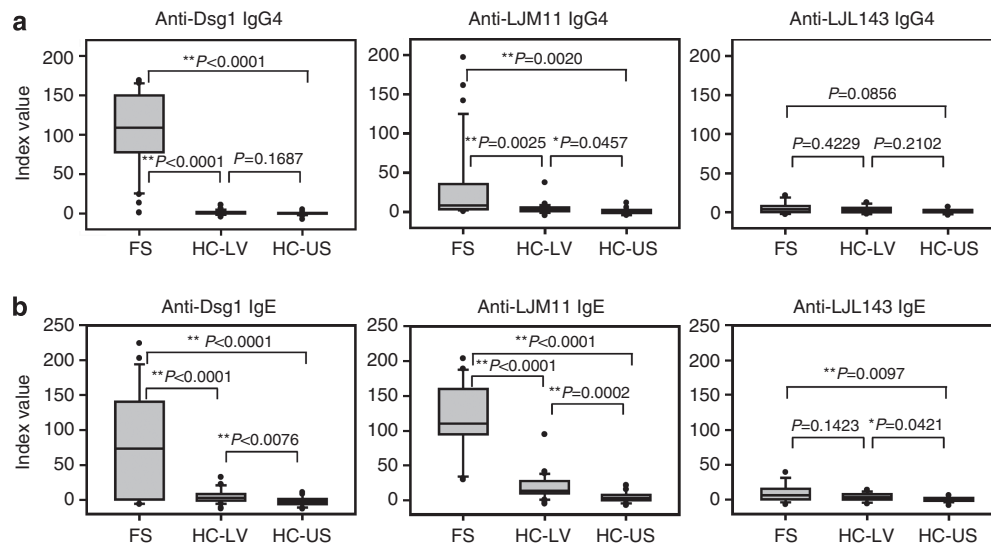


Figure 1. Fogo selvagem (FS) patients have significantly higher IgG4 (a) and IgE (b) anti-Dsg1 and anti-LJM11 antibodies compared with normal control individuals from FS and non-FS endemic regions. Anti-desmoglein 1 (anti-Dsg1) (a) and anti-LJM11 (b) ELISA were conducted as described (Qian *et al.*, 2007; 2011, 2012) using SuperSignal ELISA Pico (for IgG4) and Femto (for IgE; Thermo Fisher, Rockford, IL) as substrate. The relative light units were measured by LMax II 384 Luminometer (Molecular Devices, Sunnyvale, CA). Horseradish peroxidase conjugated mouse anti-human IgE (clone HP6029, Hamilton and Morrison, 1993) (SouthernBiotech, Birmingham, AL) was used as secondary antibody for IgE ELISA. The levels of IgG4 or IgE anti-Dsg1 or anti-LJM11 are expressed as index values (Amagai *et al.*, 1999; Qaqish *et al.*, 2009). The horizontal lines within the box plots indicate the median values. The P -values of the comparisons between each group pair are indicated. Statistical significance between any two groups is assessed by the t -test. Significant difference is marked by * ($P < 0.05$) or ** ($P < 0.01$), and P -values are indicated.

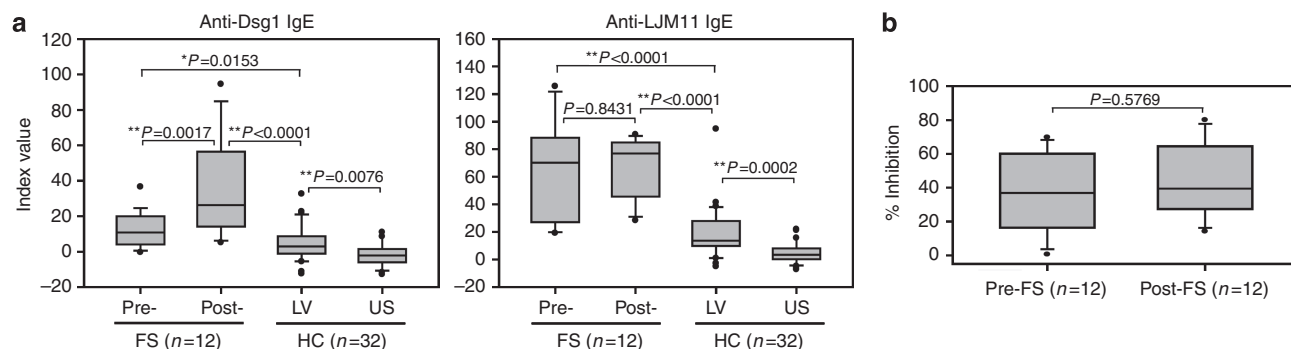


Figure 2. IgE antibodies among individuals before or after their onset of fogo selvagem (FS). (a) Anti-desmoglein 1 (anti-Dsg1) and anti-LJM11 IgE levels among pre-FS and post-FS individuals were determined by ELISA and compared with IgE and IgG4 anti-Dsg1 and LJM11 of healthy controls (HC) from both FS endemic region (Limao Verde in Brazil, LV) and non-FS endemic region (US). (b) The binding of serum IgE antibodies from pre-FS and post-FS individuals to LJM11 is inhibited by recombinant human Dsg1. IgE anti-LJM11 ELISA is conducted in the presence and absence of Dsg1. The percentage of inhibition is determined by the reduction in relative light units (RLUs) in the presence of Dsg1 ($50 \mu\text{g ml}^{-1}$) divided by RLUs without Dsg1 for each serum sample. Statistical significance between any two groups is assessed by the t -test. Significant difference is marked by * ($P < 0.05$) or ** ($P < 0.01$), and P -values are indicated.

in Brazil (HC-LV), and non-FS endemic region, United States ($n = 32$; HC-US), were tested by ELISA for their reactivity with Dsg1, LJM11, and LJL143, which is another major component of SGLL. As expected, FS patients have significantly higher levels of IgG4 anti-Dsg1 autoantibodies compared with HC-LV and HC-US (Figure 1a, left panel).

Similarly, FS patients also have significantly higher IgG4 anti-LJM11 antibodies compared with HC-LV and HC-US groups (Figure 1a, middle panel). There is no significant difference between the levels of anti-LJL143 IgG4 antibodies in the sera of FS patients and the two control groups (Figure 1a, right panel), suggesting that LJM11 is the main

component from SGLL recognized by IgG4 antibodies from FS patients. Because of the close association of the IgE and IgG4 development and our previous finding that FS patients have significant levels of IgE and IgG4 anti-SGLL (Qian *et al.*, 2012), it is expected that FS patients may also have higher IgE anti-Dsg1 and anti-LJM11 compared

with HC-LV and HC-US. As shown in Figure 1b, FS patients have significantly higher IgE anti-Dsg1 (Figure 1b, left panel) as we previously reported (Qian *et al.*, 2011, 2012) and also have significantly higher levels of IgE anti-LJM11 antibodies (Figure 1b, middle panel) compared with both HC-LV and HC-US. Importantly, HC-LV sera have higher levels of IgE anti-LJM11 antibodies compared with those from HC-US (Figure 1b, middle panel). The anti-LJL143 IgE levels are overall generally much lower (about 10 times lower) in FS, HC-LV, and HC-US groups as compared with anti-LJM11 IgE levels in the same group (Figure 1b, middle and right panel). These findings suggest that the FS endemic area of LV where the sera of FS and HC-LV originated from, and where bites by *Lutzomyia longipalpis* are prevalent, may harbor environmental factors that direct the development of these antibodies in FS endemic regions.

Our findings that LV inhabitants and FS patients show significant levels of IgE anti-LJM11 antibodies suggest that FS patients during the pre-clinical stage of the disease (pre-FS) may also exhibit elevated levels of these IgE antibodies. It is known that pre-FS would eventually develop pathogenic IgG4 autoantibodies and clinical FS (post-FS) (Qaqish *et al.*, 2009), and IgG4 antibody development lags behind the IgE response. Hence, individuals at risk to develop FS may develop IgE and IgG4 responses when exposed to LJM11 during the repeated bites of sand flies. To test this hypothesis, 12 FS patients whose serum samples were collected prior to the onset of clinical FS (1–4 years) and after their onset of FS were studied for anti-Dsg1 and anti-LJM11 IgE activity. The HC-LV and HC-US were also included as controls. As shown in Figure 2a (left panel), pre-FS and post-FS individuals exhibit higher levels of IgE anti-Dsg1 compared with the control groups. The right panel of Figure 2a shows that these pre-FS and post-FS individuals also have significantly higher levels of IgE anti-LJM11 as compared with HC-LV and HC-US. These results suggest that the IgE antibodies

against Dsg1 and LJM11 develop before the onset of FS among susceptible individuals from this endemic area. In addition, similar to IgG4 antibodies in FS, IgE anti-LJM11 and anti-Dsg1 antibodies from both pre-FS and post-FS are also cross-reactive, as their IgE binding to LJM11 can be inhibited by Dsg1 auto-antigen (Figure 2b). Notably, pre-FS individuals have significantly lower levels of IgE anti-Dsg1 compared with that from post-FS patients (Figure 2a, left panel), suggesting that anti-LJM11 IgE develops prior to that of IgE autoantibodies.

Considering that IgG4 and IgE antibody responses to allergens are closely linked, it is likely that the generation of IgE anti-LJM11, introduced by sand fly bites, results in the development of IgE and IgG4 to this antigen that cross-reacts with Dsg1 and subsequently leads to clinical FS. The definitive evolution and association of IgG4 and IgE antibody responses in FS require further analysis of the CDR3 sequences of these two classes of antibody populations. Our current findings suggest that LJM11 might be the initial target of the IgE response in FS susceptible individuals; hence, those individuals with higher levels of anti-LJM11 IgE may have a higher risk to develop FS. It may provide a basis for the possible employment of IgE as an early predictor of FS.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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