

IgE, IgM, and IgG4 Anti-Desmoglein 1 Autoantibody Profile in Endemic Pemphigus Foliaceus (Fogo Selvagem)

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TO THE EDITOR

An endemic form of *pemphigus foliaceus* (PF) exists and is virtually exclusive to certain inland states of Brazil, where it is known as fogo selvagem (FS; Diaz *et al.*, 1989b). FS shows similar clinical, histological, and immunological features to those observed in nonendemic PF (Stanley *et al.*, 1986; Diaz *et al.*, 1989a). The etiology of FS is unknown, but blister formation is mediated predominantly by IgG4 (Rock *et al.*, 1989). Moreover, a progression from preclinical to clinical disease is associated with a sharp rise in IgG4 anti-desmoglein 1 (Dsg1; Warren *et al.*, 2003), and these autoantibodies are predictors of FS (Qaqish *et al.*, 2009). Emergence of self-reactive IgG4 anti-Dsg1 in FS is a mystery; however, there are some promising circumstantial clues that point toward the etiology of the disease. For example, the antigen-selected nature of anti-Dsg1 (Qian *et al.*, 2009) and the presence of IgM anti-Dsg1 in healthy individuals in endemic areas of FS (Diaz *et al.*, 2008) strongly suggest a recent or an ongoing exposure of settlers in these endemic regions to an environmental antigen(s) (Aoki *et al.*, 2004; Diaz *et al.*, 2004, 2008).

IgG4 and IgE antibodies develop in individuals chronically exposed to environmental allergens or during immunotherapy of patients with allergic diseases (Lichtenstein *et al.*, 1968; Muller, 2005). Little is known about the IgE anti-Dsg1 response in FS, but recently Nagel *et al.* (2009) have reported that IgE anti-desmoglein 3 (Dsg3) correlated with disease activity in pemphigus vulgaris (PV) patients. In this investigation, we have evaluated

the IgE, IgM, and IgG4 anti-Dsg1 responses in a large cohort of FS patients and control individuals. A total of 558 sera were tested for IgE anti-Dsg1. Patient cohorts included 143 FS patients (Brazil), 39 nonendemic PF patients (United States and Japan), and 59 PV patients (United States and Japan). Healthy control (HC) cohorts included 161 healthy individuals living in the endemic areas of FS (Brazil), 57 healthy individuals from nonendemic areas (Brazil), and 99 healthy individuals from United States ($n=76$) and Japan ($n=23$). Dsg1-specific ELISA were carried out as described (Qian *et al.*, 2007) with a minor modification, i.e., the use of biotin-labeled mouse anti-human IgE and horseradish peroxidase-conjugated streptavidin (SouthernBiotech, Birmingham, AL). The levels of IgE anti-Dsg1 were expressed as index value units as reported by Amagai *et al.* (1999) and Diaz *et al.* (2008; Qaqish *et al.*, 2009).

The distribution of IgE index values in sera from FS, PF, and healthy individuals from Brazilian, Japanese, and US donors is shown as box plots (Figure 1a). The statistical comparisons of these groups are shown in Table 1. The IgE anti-Dsg1 index values in the FS group were higher than in the other sets, whereas HCs from US and Japan showed the lowest values. The differences in means between the FS group and all other groups tested were statistically significant at the 0.05 level. The difference between HCs from endemic regions of Brazil and nonendemic regions of Brazil was not significant. However, the difference between HCs from Brazil and United States/Japan was statistically significant.

The significantly different IgE anti-Dsg1 levels between the FS and the PF groups in United States/Japan indicate that the IgE anti-Dsg1 response in FS is a distinctive serological feature of this disease. The significant difference in IgE anti-Dsg1 between HCs in Brazil (endemic and nonendemic) and HCs in United States/Japan needs to be explored in the future. The lack of difference between HC endemic Brazil and HC nonendemic is likely explained by the fact that these groups share certain living conditions, and perhaps environmental antigens. However, these antigens may not be present in the United States and Japan.

We then analyzed the correlation between the IgE and IgG4 anti-Dsg1 responses in 143 FS and 317 HC individuals. Figure 1b shows a plot of IgE against IgG4 anti-Dsg1, with superimposed regression lines, in the FS (red) and HC groups (blue). We found a correlation between IgE and IgG4 in the FS group (Spearman's correlation $r=0.32$, $P<0.001$), and less correlation in the HC group ($r=0.15$, $P=0.07$), suggesting that the generation of these autoantibodies in FS may be linked and arising because of sensitizing to a common environmental allergen. These patterns of IgG4 and IgE responses are similar to those observed in allergic patients. Ongoing studies may demonstrate a stronger correlation between IgE and IgG4 anti-Dsg1 systems when testing sera from patients at different clinical and immunological stages of evolution.

To evaluate the humoral IgM, IgG4, and IgE anti-Dsg1 responses in FS and HC groups, we extracted data on IgM and IgG4 anti-Dsg1 for the same subjects utilized in previous publications (Diaz *et al.*, 2008; Qaqish *et al.*, 2009). A receiver operating characteristic

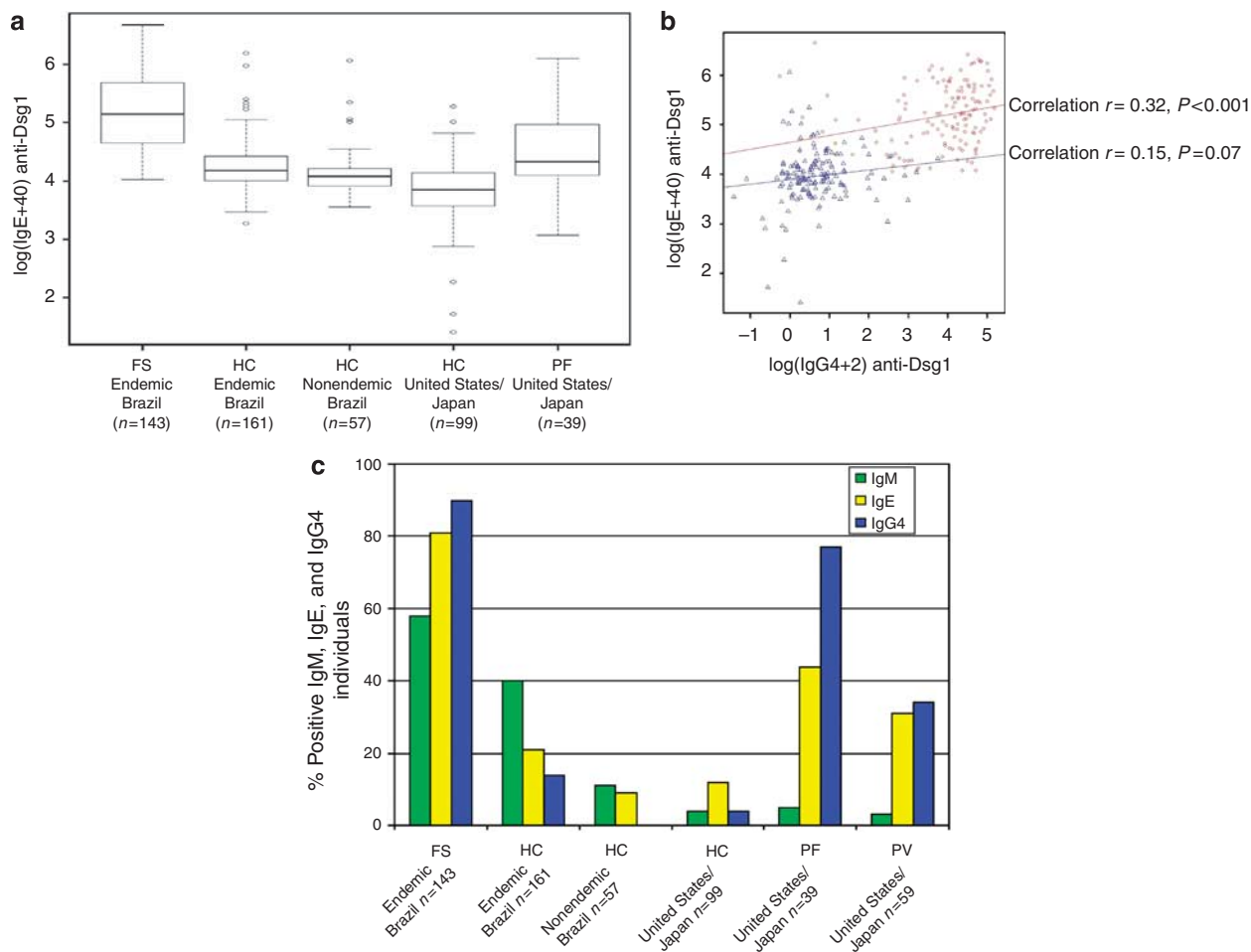


Figure 1. IgE and IgG4 anti-desmoglein 1 (Dsg1) in fogo selvagem (FS) patients and control groups. (a) Box plots of IgE anti-Dsg1 in FS and pemphigus foliaceus (PF) patients and healthy controls (HCs). The horizontal lines within the box plots indicate the median values; the lower and upper ends of the boxes represent the 25th and 75th percentiles. (b) The correlation between anti-Dsg1 IgE and IgG4 in FS patients and healthy controls. Blue triangles and blue line indicate healthy controls; red circles and red line indicate FS patients. (c) IgM, IgE, and IgG4 anti-Dsg1 in FS, PF, pemphigus vulgaris (PV), bullous pemphigoid, and HCs. Percentages of positive anti-Dsg1 sera for each group are shown in green bars (IgM), yellow bars (IgE), and blue bars (IgG4).

Table 1. Comparison of the IgE anti-Dsg1 autoantibodies in FS and control groups

Comparison	Difference between means of index value	Simultaneous 95% confidence limit	P-value
FS versus PF United States/Japan	0.69	0.42 0.96	0.05
FS versus HC endemic area Brazil	0.90	0.73 1.07	0.05
FS versus HC nonendemic area Brazil	1.01	0.78 1.25	0.05
FS versus HC United States/Japan	1.32	1.13 1.51	0.05
PF United States/Japan versus HC United States/Japan	0.63	0.35 0.91	0.05
HC endemic area Brazil versus HC nonendemic area Brazil	0.12	-0.11 0.34	NS
HC United States/Japan versus HC endemic area Brazil	-0.42	-0.61 -0.23	0.05
HC United States/Japan versus HC nonendemic area Brazil	-0.31	-0.55 -0.06	0.05

Abbreviations: Dsg1, desmoglein 1; FS, fogo selvagem; HC, healthy control; NS, nonsignificant comparison; PF, pemphigus foliaceus. Analysis of differences between groups used one-way analysis of variance with adjustment for multiple comparisons by Tukey's studentized range test. Analysis was done on log(IgE+40) and log(IgG4+2) as these transformed variables were approximately normally distributed. Analysis was done in the R 2.9 software (R Foundation for Statistical Computing, Vienna, Austria).

(ROC) curve for IgM, IgE, and IgG4 anti-Dsg1 index values was used to determine a cutoff point for sensitivity

and specificity as described (Diaz *et al.*, 2008; Qaqish *et al.*, 2009). The percentages of positive sera for IgM, IgE,

and IgG4 anti-Dsg1 in the different groups were plotted and shown in Figure 1c. It is apparent that the triple

response of IgM, IgG4, and IgE anti-Dsg1 is much higher in FS patients than in HC from the same endemic regions and HC from nonendemic regions of Brazil and United States/Japan (χ^2 , $P < 0.05$, for IgM, IgE, and IgG4 anti-Dsg1). The percentages of positive individuals for IgM and IgE anti-Dsg1 were higher in the FS group (58 and 81%, respectively) than in all other groups (χ^2 , $P < 0.05$). As expected and also shown in Figure 1c, IgG4 anti-Dsg1 was detected in a large number of FS, PF, and PV patients (90, 77, and 33% respectively), which is a known feature of these diseases. These findings suggest a persistent antigenic stimulation of the immune system of individuals living in the endemic areas of FS.

In summary, our findings further support the notion that the anti-Dsg1 response in FS patients may be initiated by sensitization to an environmental allergen(s). The crossreactive IgE, IgM, and pathogenic IgG4 anti-Dsg1 response may be the serological markers of these immunological responses.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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