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Endemic Pemphigus Foliaceus: Towards Understanding Autoimmune Mechanisms of Disease Development

Giovanni Di Zenzo¹, Giovanna Zambruno¹ and Luca Borradori²

Fogo selvagem is an endemic form of pemphigus foliaceus (EPF) found in Brazil. Environmental and genetic factors are thought to contribute to the disease, which is associated with pathogenic IgG4 autoantibodies against the desmosomal cadherin desmoglein 1. In this issue, as an additional framework to understand autoimmune mechanisms in EPF, Flores *et al.* have investigated whether fogo selvagem patients and healthy individuals from endemic areas develop autoantibody responses against other desmosomal cadherins and E-cadherin.

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Pemphigus comprises a group of potential life-threatening autoimmune blistering diseases of the skin and mucous membranes. This group of diseases is characterized by autoimmune responses

directed specifically against two transmembrane components of desmosomes, desmoglein (Dsg) 3 and Dsg1, adhesion molecules of the cadherin family. Additional targeted antigens have been

identified recently, the pathogenic significance of which has not been demonstrated conclusively. The two major forms of the disease—pemphigus vulgaris (PV) and pemphigus foliaceus (PF)—differ clinically. PF develops as either sporadic disease occurring all over the world or in an endemic form. The latter is found mainly in certain areas of Brazil, where the disease is known as fogo selvagem (wildfire), as well as in other South and Central American countries (Diaz *et al.*, 1989; Abr  u-Velez *et al.*, 2003). Finally, an endemic form of PF has been also described in North Africa (Kallel Sellami *et al.*, 2004). Although endemic PF (EPF) and the sporadic form share similar clinicopathological features and the presence of pathogenic IgG anti-Dsg1 antibodies, fogo selvagem has peculiar characteristics.

Fogo selvagem and its unique epidemiology

Over the past 25 years, Diaz and the Cooperative Group on Fogo Selvagem Research have carried out a series of remarkable epidemiologic and immunologic studies on a specific population of Amerindians living in the Terena reservation of Limao Verde in Mato Grosso do Sul, Brazil. In this population, the prevalence of EPF is approximately 3.5%. Most of the affected patients are children and young adults, who often work outdoors and live in forested areas near rivers and streams, in poor hygienic and housing conditions. A strong relationship has been found between the occurrence of fogo selvagem and infestation with hematophagous insects such as *Simulium* (also called black fly; Aoki *et al.*, 2004). Strikingly, up to 80% of subjects living in these areas who suffer from hematophagous vector-borne parasitic diseases such as onchocerciasis, leishmaniasis, and Chagas disease also exhibit anti-Dsg1 antibodies (Diaz *et al.*, 2004). Patients with fogo selvagem show circulating IgM and IgE anti-Dsg1 antibodies, which have been thought to result from continuous antigenic stimulation (Qian *et al.*, 2011). Together, these observations have supported the idea that environmental factors have a key role in triggering EPF in individuals with certain HLA susceptibility genes, especially

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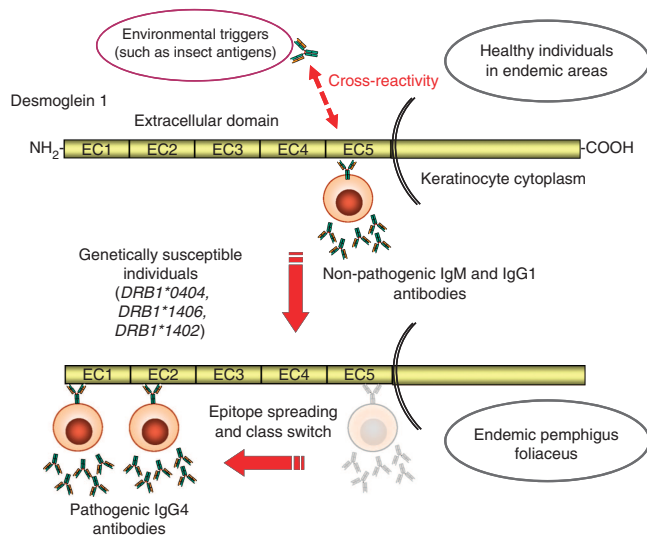


Figure 1. Current etiopathogenic model for endemic pemphigus foliaceus.

*DRB1*0404* and *DRB1*1402* or *DRB1*1406* (Moraes *et al.*, 1997).

Fogo selvagem and dissection of the autoimmune mechanisms leading to overt disease in endemic areas

Warren *et al.* have previously shown that a high percentage of healthy individuals (up to 55%) living in fogo selvagem endemic areas have low levels of anti-Dsg1 antibodies, and that onset of EPF is associated with anti-Dsg1 antibody subclass switching from IgG1 to IgG4. The latter represents the predominant and pathogenic autoantibodies found in sporadic PF (Warren *et al.*, 2003). Furthermore, overt disease is accompanied by the emergence of pathogenic antibodies directed specifically against the N-terminal region of Dsg1 ectodomain (EC1 and EC2 subdomains; Li *et al.*, 2003), whereas patients in the preclinical stage and healthy individuals from endemic areas possess IgG1-circulating autoantibodies that recognize nonpathogenic epitopes in the C-terminal portion of the Dsg1 ectodomain (EC5 subdomain). On the basis of these findings, a model has been proposed in which an environmental antigen showing molecular mimicry with Dsg1 triggers a nonpathogenic IgG1 antibody response to the EC5 subdomain of Dsg1. Overt disease occurs subsequently only in genetically susceptible individuals, and it is associated with both a subclass switch leading to the production of pathogenic

IgG4 anti-Dsg1 antibodies and epitope spreading, with the development of reactivity against the EC1 and EC2 domains of Dsg1 (Figure 1). Although the epidemiologic and immunologic characteristics of affected patients are not identical to those of patients with fogo selvagem, this model likely applies also to the EPF found in Tunisia (Kallel Sellami *et al.*, 2004).

Dsg1 and beyond in fogo selvagem: a role for other keratinocyte cadherins?

As an additional important element in the framework to understand autoimmune mechanisms in EPF, Flores *et al.* (2012) and the Cooperative Group on Fogo Selvagem Research have investigated whether fogo selvagem patients develop autoantibody responses against other desmosomal cadherins and E-cadherin.

The desmosomal cadherin family comprises four Dsgs (Dsg1–4) and three desmocollins (Dsc1–3), which share a common molecular organization with five extracellular subdomains, a transmembrane subdomain, an intracellular anchor subdomain, and a cytoplasmic subdomain. A similar structural organization with five extracellular subdomains is also found in classic cadherins, such as E-cadherin. Although Dsg3 and 1 are the major targets of pathogenic autoantibodies in mucosal dominant PV and PF, respectively, several studies have described autoantibody

responses to other members of the cadherin family in patients with different forms of pemphigus. Specifically, Dscs are antigenic targets in IgA pemphigus and in a subset of PV patients, although antibodies binding to Dsg4 and E-cadherin have also been detected in mucocutaneous PV and PF sera (Nagasaka *et al.*, 2004; Evangelista *et al.*, 2008; Rafei *et al.*, 2011; Tsuruta *et al.*, 2011). However, antibodies to Dsg4 in PV and PF exclusively represent cross-reactivity with a subset of anti-Dsg1 autoantibodies, and the same holds true for most PV and PF sera with autoantibodies against E-cadherin (Nagasaka *et al.*, 2004; Evangelista *et al.*, 2008).

Endemic pemphigus vulgaris prototypes the interplay of environment with susceptibility genes.

Flores *et al.*, 2012 have thus assessed IgG reactivities against Dsg1–4 and Dsc1–3, as well as E-cadherin, in a large cohort of fogo selvagem patients ($n=111$), in healthy controls living in the Limao Verde endemic region (endemic controls, $n=106$), and in normal individuals from a nonendemic area (US controls, $n=106$). By using ELISA assays, they detected antibody responses against several keratinocyte cadherins in addition to Dsg1 in EPF and in endemic control sera. Specifically, they found significantly higher index values (i) for Dsg4, Dsc3, and E-cadherin in endemic controls than in US controls; (ii) for Dsg1–3 and Dsc1 in EPF patients than in endemic controls; and (iii) for Dsg1–3, Dsc1 and 3, and E-cadherin, in EPF patients than in US controls. Anti-E-cadherin IgG antibodies were detectable in a significant percentage of sera from endemic control (58%) and EPF (42%) groups. In endemic controls, Flores *et al.* (2012) observed a striking correlation between the presence of anti-Dsg1 autoantibodies and reactivity with Dsg3, Dsg4, Dsc1–3, and E-cadherin. Furthermore, US controls showed unexplained higher reactivities against Dsg3 and Dsc2 than in the endemic controls and EPF patients, respectively. Finally, the investigators

assessed the diagnostic performance of their ELISAs. They found that the frequency of positive EPF and endemic control sera for all the tested antigens, except Dsc3 and Dsg3, was above 20%. However, given the relatively low sensitivity, none of the cadherin-based ELISAs, with the exception of the Dsg1 assay, appeared useful as a diagnostic test for EPF.

Nevertheless, some caution should be exercised with the findings of Flores *et al.* (2012). First, the reliability of the ELISA data comparisons is to some extent weakened by the high levels for negative controls and by saturated ELISA values. Second, immunoblotting and immunofluorescence studies would have been useful to support the findings obtained by ELISA, at least for selected cadherin molecules not previously tested. Finally, the authors did not conduct studies to determine whether the detected anti-Dsc1-3, anti-Dsg1-4, and anti-E cadherin antibodies were independent antibody responses or represented cross-reactivities. However, the same group has provided evidence previously that most of the anti-E-cadherin antibodies cross-react with Dsg1 in pemphigus patients because of the high homology between these two molecules, although others represent independent antibodies (Evangelista *et al.*, 2008). Furthermore, the reactivity detected previously for Dsg4 in pemphigus patients has been attributed to cross-reactivities for a subset of anti-Dsg1 antibodies (Nagasaka *et al.*, 2004).

On the basis of their findings, Flores *et al.* (2012) put forward novel speculation about the pathogenesis of EPF. The earliest response in healthy individuals from endemic areas, which may be environmentally triggered, might be directed against one of the keratinocyte cadherins that is recognized differently in the endemic and normal controls, such as E-cadherin. This initial response is followed by the production of autoantibodies against other keratinocyte cadherins, most likely Dsg1-3, Dsc1, and Dsc3. This immune response may either reflect cross-reactivity and/or be due to intermolecular epitope spreading, subsequently leading to the generation of highly specific and

pathogenic IgG4 autoantibodies that are responsible for overt disease.

Qian *et al.* (2012) have recently reported that salivary gland antigens from the sand fly, which is a vector for leishmaniasis, are recognized by IgG4 and IgE antibodies from EPF patients. Intriguingly, these investigators demonstrated further that mice immunized with one of these fly antigens produce anti-Dsg1 antibodies (Qian *et al.*, 2012). Although there is no sequence similarity between Dsg1 and the fly antigens, cross-reactivity may occur in response to the presence of the same conformational epitopes on both molecules. Surprisingly, the antibody titers against the salivary gland antigens in endemic controls were similar to those detected in the US controls. It is conceivable that the fly salivary gland epitope mimics a pathogenic epitope in the EC1/EC2 subdomains of Dsg1, resulting in the induction, in the endemic patients, of a cross-reactive anti-Dsg1 immune response that, in genetically predisposed individuals, leads directly to overt disease. Hence, it would be of interest to assess whether the recognized salivary gland epitopes (and other potential triggering antigens) are shared by keratinocyte cadherins and, in particular, by E-cadherin. The latter was suggested by Flores *et al.* (2012) to act as possible link between the immune response of endemic controls and that of EPF patients.

Interplay of genetic and environmental factors in the development of endemic PF

The strong associations with distinct HLA class II alleles reported in both Brazilian and Tunisian EPF patients (Abida *et al.*, 2009) implies that genetic factors influence the development of EPF. In this context, the report by Flores *et al.* (2012) does not provide new data concerning the relative contributions of environmental and genetic factors to the autoantibody responses in EPF patients or in healthy individuals living in endemic areas. The predisposing role of HLA and other susceptibility genes in the complex pathogenesis of EPF requires further characterization.

Recently, we isolated several anti-Dsg3 pathogenic IgG monoclonal antibodies from PV patients. These

autoantibodies carried high levels of somatic mutations in complementary determining regions, consistent with antigen selection. Remarkably, binding of antibodies to Dsg3 was lost when somatic mutations were reverted to the germline sequence, suggesting the role of a different antigen as the primary trigger in the development of the immune response leading to PV (Di Zenzo *et al.*, 2012). Our findings are, thus, in line with available data in EPF, indicating that autoreactivity to Dsg1 could be generated following the recognition of a disease-triggering antigen most likely unrelated to Dsg1. In addition, a recent study by Qian *et al.* (2009) showed that hybridomas established from B cells from an EPF patient before and after disease onset share multiple replacement mutations. This observation strongly suggests that antigen selection occurs well before the onset of disease. Hence, characterization of the antigenic targets of germline antibodies, obtained through the reversion of somatic mutations of anti-Dsg1 monoclonal antibodies isolated from EPF patients, may provide novel insights into the pathogenesis of EPF and other human autoimmune diseases.

It is hoped that the Cooperative Group on Fogo Selvagem Research, as well as other laboratories, will continue their efforts with epidemiologic, genetic, and immunologic studies aimed at further dissecting the mechanisms leading to EPF. EPF has emerged as a prototypic model for the study of the relationship and interplay between environmental triggering factors and susceptibility genes, which will prove useful and important for understanding a variety of other organ-specific autoimmune diseases.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Activation of the Unfolded Protein Response in Vitiligo: The Missing Link?

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Vitiligo is characterized by a substantial loss of functional melanocytes in the epidermis and sometimes in hair follicles. Genetic and pathophysiological studies have provided strong evidence that vitiligo is a polygenetic, multifactorial disorder. The key roles of oxidative stress within melanocytes and anti-melanocyte immune responses have been addressed in many studies, but the relationship between these mechanisms remains unclear. In this issue, Toosi *et al.* report the upregulation of IL-6 and IL-8 after the activation of the unfolded protein response (UPR) following exposure of melanocytes to phenols. Their results shed light on the missing link between oxidative stress and immune responses in vitiligo.

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Oxidative stress in vitiligo

Vitiligo melanocytes long have been known to have a low replication rate in culture, requiring catalase supplementation for their growth and showing structural aberrations, such as dilated endoplasmic reticulum (Boissy *et al.*, 1991). High levels of hydrogen peroxide have been found in the vitiligo epidermis (Schallreuter *et al.*, 1999). Indeed, vitiligo melanocytes are the theater of a redox balance loss because of high levels of toxic catechol metabolites, high free-radical levels, and defective antioxidant machinery (Dell'anna and Picardo, 2006). In culture, vitiligo melanocytes are highly susceptible to chemical and physical oxidative stress. The overactivation of some sources of reactive oxygen species (ROS), the role of NADPH oxidase in ROS formation caused by extracellular and intracellular factors/conditions, and deficiencies in one or more of the reducing agents and detoxifying enzymes are responsible for this redox homeostasis impairment. Dysfunction of catalase, superoxide dismutase, glutathione peroxidase, thioredoxin peroxidase, thioredoxin reductase, and

γ-glutamyl transpeptidase has been reported extensively for vitiligo. The first evidence supporting the concept that stressed melanocytes could activate an immune response was provided by the identification of activated dendritic cells after intrinsic damage to melanocytes due to 4-tertiary butyl phenol exposure (Kroll *et al.*, 2005).

Vitiligo, an autoimmune disorder

Studies of the infiltrate in perilesional epidermis of vitiligo skin reveal predominantly CD8+ cells (Lili *et al.*, 2012). A recent mouse model of vitiligo, with a phenotype similar to that observed in humans, was developed using melanocyte-specific CD8+ T cells (Harris *et al.*, 2012). Interestingly, in this model, the inhibition of IFN-γ with antibody prevents the development of depigmentation. Th1 and, more recently, the Th17 cytokine environment have been implicated in the pathophysiology of vitiligo (Wang *et al.*, 2011; Kotobuki *et al.*, 2012). Dermal dendritic cells with activated inflammasomes have been found at the edges of vitiligo lesions, and they could participate in the Th17 activation (Wang

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