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# Pathogenic IgG4 autoantibodies from endemic pemphigus foliaceus recognize a desmoglein-1 conformational epitope

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ABSTRACT

Fogo Selvagem (FS), the endemic form of pemphigus foliaceus, is mediated by pathogenic IgG4 autoantibodies against the amino-terminal extracellular cadherin domain of the desmosomal cadherin desmoglein 1 (Dsg1). Here we define the detailed epitopes of these pathogenic antibodies. Proteolytic footprinting showed that IgG4 from 95% of FS donor sera (19/20) recognized a 16-residue peptide ($A^{129}$LNSMGQDLERPLELR$^{144}$) from the EC1 domain of Dsg1 that overlaps the binding site for an adhesive-partner desmosomal cadherin molecule. Mutation of Dsg1 residues $M^{133}$ and $Q^{135}$ reduced the binding of FS IgG4 autoantibodies to Dsg1 by ~50%. Molecular modeling identified two nearby EC1 domain residues ($Q^{82}$ and $V^{83}$) likely to contribute to the epitope. Mutation of these residues completely abolished the binding of FS IgG4 to Dsg1. Bead aggregation assays showed that native binding interactions between Dsg1 and desmocollin 1 (Dsc1), which underlie desmosome structure, were abolished by Fab fragments of FS IgG4. These results further define the molecular mechanism by which FS IgG4 autoantibodies interfere with desmosome structure and lead to cell-cell detachment, the hallmark of this disease.
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## 1. Introduction

Desmosomal cadherins, including desmogleins (Dsg1-4) and desmocollins (Dsc1-3), are desmosomal adhesion molecules that play a critical role in desmosome-mediated intercellular adhesions between epidermal keratinocytes [1]. Autoantibodies targeting Dsg1 and Dsg3 cause pemphigus foliaceus (PF) and pemphigus vulgaris (PV), a group of serious cutaneous blistering diseases [2]. Fogo Selvagem (FS), the endemic form of PF, is a cutaneous autoimmune blistering disease that exhibits geographic clustering in certain regions of Brazil [3,4]. FS and PF are characterized by intraepidermal subcorneal blisters due to cell detachment, a process known as acantholysis [5]. It is well established that PF and FS patients possess IgG anti-epidermal autoantibodies, which correlate with disease-extent and activity [6,7]. These autoantibodies bind specifically to Dsg1 [8] and do not bind other desmosomal cadherins, such as Dsg2, Dsg3, Dsg4 or Dsc1-3 [9–12]. Moreover, these autoantibodies are predominantly restricted to the IgG4 isotype [13], conformation- and calcium-dependent [14–17], carbohydrate-independent [17–19] and pathogenic [7,13]. Both the whole IgG fractions and the $F(ab)_2$ or monovalent Fab fragments

*Abbreviations:* FS, fogo selvage; Dsg, desmoglein; Dsc, desmocollin; EC, extracellular; PF, pemphigus foliaceus; HEK, human embryonic kidney.

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from FS patients faithfully reproduce the human disease in neonatal micepassive transfer experiments [20,21]. Additionally, the appearance of FS IgG4 anti-Dsg1 autoantibodies in individuals living in endemic areas of FS heralds the onset of disease by months or even years, i.e., these autoantibodies serve as disease predictors [22].

It was previously shown that the immunoreactive region of Dsg1 is located within the N-terminal EC domains of Dsg1 [23,24]. Later, our group showed that IgG from FS patients preferentially binds to epitopes located on the EC1 and EC2 domains of Dsg1 [25]. However, these early studies were carried out using mainly domain-swapped segments of Dsg1 on Dsg3 backbones [24,25]. Consequently, the fine specificity of the IgG4 anti-Dsg1 autoantibodies on Dsg1 had not been determined until now. Attempts to define Dsg1 epitopes may be relevant in understanding the molecular aspects of acantholysis and may provide new approaches to search for environmental antigens linked to FS.

In this investigation, we generated hybrid molecules encompassing the EC1 domain of Dsg1 grafted on three backbone (bb) carriers made of the EC2-EC5 domains of Dsg3 (Dsg1-EC1/Dsg3 bb), Dsg4 (Dsg1-EC1/Dsg4 bb) and desmocollin-1 (Dsc1) (Dsg1-EC1/Dsc1 bb). An additional hybrid was generated by inserting the EC1 domain of Dsg4 on a backbone of the EC2-EC5 domain of Dsg1 (Dsg4-EC1/Dsg1 bb). Using an enzyme-linked immunosorbent assay (ELISA), we showed that IgG4 from FS patients bound the Dsg1-EC1 in all three carriers; however, the hybrid of Dsg4-EC1 on the Dsg1 backbone was not reactive. Further, IgG4 autoantibodies bound specifically to a Dsg1-EC1/Dsg3 bb or Dsg1-EC1/Dsg4 bb Sepharose matrix and the eluted IgG4 autoantibodies were pathogenic when passively transferred into neonatal mice. We next tested purified FS IgG4 fractions from 20 FS patients by proteolytic footprinting [26–28] using recombinant Dsg1 ectodomain. Affinity-purified IgG4 from 19 out of 20 patients recognized a 16-amino acid peptide ($A^{129}$LNSMGQDLERPLELR$^{144}$); notably, residue $A^{129}$ within the peptide lines the Trp 2 acceptor pocket, which is critical for adhesive binding. Site-directed mutagenesis of residues $M^{133}$ and $Q^{135}$ of the Dsg1-EC1 epitope decreased the binding of IgG4 anti-Dsg1 autoantibodies by approximately 50%. Additional mutations of residues $Q^{82}$ and $V^{83}$ of the Dsg1-EC1 epitope further decreased the binding of IgG4 autoantibodies to baseline levels. Most importantly, Fab fragments of FS IgG4 blocked heterophilic interactions of Dsg1 and Dsc1 in aggregation assays [29]. These findings further advance the understanding of the basic desmosomal cadherin interactions and how pathogenic autoantibodies from PF/FS patients specifically interfere with such interactions, possibly leading to cell detachment and blister formation.

## 2. Materials and methods

### 2.1. Sources of human FS sera

Well-characterized sera from 20 patients with classic FS, as defined by clinical, histological, immunological and epidemiological criteria [3–5], were available for epitope mapping studies. FS patients showed a widespread blistering eruption located on the trunk and extremities. Some patients showed facial and scalp lesions as well. Biopsies from these lesion showed typical subcorneal vesicles. Normal human sera from individuals living in endemic areas of FS in Brazil (n = 30), and non-endemic areas of FS in Brazil (n = 30) and the USA (n = 30) were also tested. Sera of FS patients and controls were collected following IRB policies from the University of North Carolina at Chapel Hill (USA) and the University of São Paulo (Brazil). The sera are kept frozen at −30 °C at the UNC Dermatology Research Laboratories.

### 2.2. Purification of IgG4 autoantibodies from FS sera

IgG4 autoantibodies were purified from the sera of FS patients by affinity chromatography using Capture Select® human IgG4 affinity matrix (BAC BV, Leiden, The Netherlands). The Capture Select® human IgG4 affinity matrix contains a 12kD llama antibody fragment that specifically recognizes human IgG4 without cross-reacting with other human IgG subclasses 1, 2 or 3. Briefly, FS serum was loaded onto the matrix and later washed with PBS, pH = 7.4. The matrix was eluted with 0.1 M Glycine, pH = 3.0. Bound and unbound fractions were tested by quantitative sandwich ELISA, developed in our laboratory using goat $F(ab)_2$ anti-human IgG (Fab-specific) as the capture antibody, and monoclonal anti-human IgG or IgG subclass horseradish-peroxidase conjugates as detecting antibodies. Both fractions were dialyzed against PBS at pH = 7.4, concentrated by ultrafiltration and stored at −20 °C. The values of IgG and IgG subclasses were expressed as percentages from the total IgG loaded onto the matrix. The eluted fraction contained 97% IgG4. The unbound fraction contained 92% IgG1, with small amounts of IgG2 and IgG3. In addition, total IgG was purified by Protein G-affinity chromatography using HiTrap Protein G HP cartridge (GE Healthcare, Pittsburgh, PA, USA), from a normal individual living in endemic areas of FS. Normal human IgG4 kappa utilized as control was purchased from Sigma-Aldrich (Saint Louis, MO, USA).

### 2.3. Preparation of Fab fragments normal human and FS IgG4

4 mg of affinity purified IgG4 from a normal human and a FS patient (FS-1) were digested with papain according to PierceTM Fab Preparation Kit, Pierce Biotechnology(Rockford Illinois, USA), to generate IgG4 Fab fragments. The IgG4 Fab samples were concentrated to 1 mg/ml using Amicon Ultra-0.5 ml Centrifugal Filters Ultracel -3K (Merk Millipore Ltd Tullagreen Carrightwohill Co Cork, Ireland) and exchanged to PBS buffer at pH 7.5. The Fab fragments from normal human serum and FS-1 were tested by indirect IF against human skin cryosections, immunoblotting and ELISA using Fab and Fc specific sera. Additionally, the Fab fragments were tested by passive transfer experiments on the neonatal mouse model and the aggregation assay of bead coated with Dsg1 and Dsc1 as described by Harrison et al. [29].

### 2.4. Plasmid constructs of Dsg1, Dsg3, Dsg4 and Dsc1

The ectodomain of human Dsg1 and human Dsg3 were already available in our laboratory [25]. Briefly, cDNA encoding the entire extracellular domain of Dsg1, including a COOH-terminal Histidine-tag, was subcloned into the baculovirus transfer vector pVL1393 (BD Biosciences, San Jose, CA, USA) and later sequenced to verify sequence integrity. The cDNA encoding the entire extracellular domain of human Dsg4 harboring an E-tag and His-tag was cloned into the pQE-TriSystem vector (QIAGEN, Inc., Chatsworth, CA, USA), as previously described [11]. Dsg4 cDNAs [11] and desmocollin-1 (Dsc1) cDNAs [12] were kindly provided by Dr. Masayuki Amagai (Keio University, Tokyo, Japan) and Dr. Takashi Hashimoto (Kurume University, Kurume, Japan), respectively.

### 2.5. Expression and purification of recombinant desmosomal proteins

Plasmids encoding the wild type ectodomains of desmosomal proteins and their mutants were co-transfected and expressed in a baculovirus expression system (BD BaculoGold, BD Biosciences,

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