Cardiac rhythm and pacemaking abnormalities in patients affected by endemic pemphigus in Colombia may be the result of deposition of autoantibodies, complement, fibrinogen, and other molecules

Ana Maria Abreu Velez, MD, PhD, DSc,* Michael S. Howard, MD,*
Jorge Enrique Velazquez-Velez, MD†

*From the *Georgia Dermatopathology Associates, Atlanta, Georgia, and †Department of Cardiology, Hospital General de Medellin, and Clinica CES Medellin, Antioquia, Colombia.*

BACKGROUND We previously showed that one-third of patients affected by endemic pemphigus foliaceus in El Bagre, Colombia (El Bagre-EPF), display autoreactivity to the heart.

OBJECTIVE The purpose of this study was to investigate rhythm disturbances with the presence of autoantibodies and correlate them with ECG changes in these patients.

METHODS We performed a study comparing 30 patients and 30 controls from the endemic area, matched by demographics, including age, sex, weight, work activities, and comorbidities. ECG as well as direct and indirect immunofluorescence, immunohistochemistry, and confocal microscopic studies focusing on cardiac node abnormalities were performed. Autopsies of 7 patients also were reviewed.

RESULTS The main ECG abnormalities seen in the El Bagre-EPF patients were sinus bradycardia (in one-half), followed by left bundle branch block, left posterior fascicular block, and left anterior fascicular block compared with the controls. One-third of the patients displayed polyclonal autoantibodies against the sinoatrial and/or AV nodes and the His bundle correlating with rhythm anomalies and delays in the cardiac conduction system ($P < .01$). The patient antibodies colocalized with commercial antibodies to desmoplakins I and II, p0071, armadillo repeat gene deleted in velo-cardio-facial syndrome (ARVCF), and myocardium-enriched zonula occludens-1-associated protein (MYZAP; Progen Biotechnik) ($P < .01$).

CONCLUSION One-third of the patients affected by El Bagre-EPF have rhythm abnormalities that slow the conduction of impulses in cardiac nodes and the cardiac conduction system. These abnormalities likely occur as a result of deposition of autoantibodies, complement, and other inflammatory molecules. We show for the first time that MYZAP is present in cardiac nodes.

KEYWORDS Autoimmune disease; Endemic pemphigus foliaceus; Rhythm abnormality; Sinus bradycardia

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Introduction

Endemic forms of pemphigus foliaceus are a unique group of autoimmune diseases, and the diseases frequently run in families.[1–4] These disorders offer an outstanding opportunity to study interactions of the environment and genetics with the immune system.[1–4] These diseases are characterized by restriction to relatively well-defined geographic regions of South and Central America and Tunisia, Africa.[1–4] We previously described a new variant of endemic pemphigus foliaceus in El Bagre, Colombia (El Bagre-EPF; pemphigus Abreu-Manu). The disease presents in gold and other mining areas polluted with mercury and other metals and metalloids, and with severe deforestation.[5–9] El Bagre-EPF occurs in several clinical and immunologic forms, including fruste, bullous, foliaceus, erythrodermic, pustular, hyperpigmented, papillomatous, and a generalized form with systemic anomalies. The milder form is localized to the skin. We have demonstrated a systemic form in about one-third of the patients that affects several organs, including the kidney. It is characterized by episodic relapses, a tendency toward chronicity, challenging treatment, and a worse prognosis compared to the localized form.[5–11] The systemic form also affects the cardiovascular system, including its neurovascular junctions.[5–7] We previously showed cardiac anomalies, including sudden death syndrome and syncope, and autoantibodies to several parts of the heart in El Bagre-EPF patients.[7] We now aim to continue investigating cardiac rhythm disturbances in these patients and to correlate them with the presence of antibodies against cardiac structures.

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Methods

We performed a pilot study testing 30 patients affected by El Bagre-EPF and 30 controls from the endemic area matched by demographics (age, sex, and weight), work activities, comorbid acute and/or chronic conditions, exposure to chemicals, socioeconomic status and income, exposure to sun, mobility and changes in residences, food intake, medications, and exposure to insect bites. We used a paired case-control study, correlating the presence of autoantibodies against the cardiac conduction system vs a lack of immunologic findings in the controls. The patients were also evaluated epidemiologically and clinically by ECG, skin biopsies (hematoxylin and eosin [H&E] staining), direct and indirect immunofluorescence (DIF, IIF), immunohistochemistry (IHC), and Airyscan confocal microscopy (CFM) staining. Only patients who met diagnostic criteria for El Bagre-EPF were included, specifically: (1) patients who displayed clinical and epidemiologic features described for this disease[5,6]; (2) patients who lived in the endemic area; (3) patients whose serum displayed intercellular staining between epidermal keratinocytes and the basement membrane zone of the skin by either DIF or IIF using fluorescein isothiocyanate–conjugated monoclonal antibodies to human total immunoglobulin (Ig) G or IgG4, as previously described elsewhere[5,6]; (4) each patient whose serum was positive by immunoblotting for reactivity against desmoglein-1 (Dsg1), as well as for plakin molecules as previously described[5–9]; (5) each patient whose serum immunoprecipitated a concanavalin A affinity-purified bovine tryptic 45-kDa fragment of Dsg1[8]; and (6) each patient whose serum yielded a positive result using an enzyme linked immunosorbent assay when screening for autoantibodies to EPF antigens.[9] Written consent was obtained from all patients, and permission was obtained from the institutional review board at Hospital Nuestra Señora de El Carmen, El Bagre, Colombia. All patient cases and controls were tested using the same methods and under the same conditions. Positive and negative controls were performed for all tests. We recorded patient demographics, weight and obesity measurements, comorbidities, work activities, walking distances to homes and workplaces, and exposure to environmental risk factors. After these measurements, "matched controls" were selected as a control group. The matched controls were deemed by these criteria to be at similar risk as the El Bagre-EPF patients for development of the disease. We also performed H&E and IHC studies for 7 autopsies of patients who had died affected by EPF. No ECG data were available for these 7 patients, and they were not part of the study group of 30 patients.

IIF and DIF

In brief, we dissected the hearts of cows and localized the sinoatrial and AV nodes, and the His–Purkinje system. We then used H&E staining and IHC to verify their nature. For IIF, we incubated 4-μm-thick antigen tissue from each node and the His–Purkinje system with patient and control sera. For DIF, we incubated skin tissue from each patient and/or control with secondary antibodies as previously described.[7–9] We incubated the slides with phosphate-buffered saline (PBS) and 3.5% paraformaldehyde. The slides were then washed twice with PBS, permeabilized using PBS with 0.1% Triton X-100, blocked with 1% normal goat serum, and washed with PBS. We then applied rabbit anti-human total IgG, IgA, IgM, κ and λ light chains, and C1q and C3 antibodies to the slides. We also used antibodies against fibrinogen and albumin. All of the preceding antibodies were obtained from Dako (Carpinteria, CA). In addition, anti-human IgE antiserum was obtained from Kent Laboratories (Bellingham, WA) and anti-human IgD antibodies from Southern Biotechnology (Birmingham, AL). The DIF slides were counterstained with DAPI (Pierce, Rockford, IL).[5–9] Several years ago, Dr. Abreu discovered new El Bagre-EPF autoantigens to several organs other than the skin. Because of the complexity of the immune response, we contacted other experts in the field, including Dr. Ernest H. Beutner in the United States, Dr. Takashi Hashimoto in Japan, and Dr. Werner W. Franke, ex-professor at the University of Heidelberg, Germany. All agreed that the data indicated new autoantigens and that the disease was unique. A few months later, the primary owner of Progen Biotechnik (Dr. Franke) illegally commercialized them without Dr. Abreu's permission. We thus used antibodies to desmoplakins 1 and 2 (DPI/II; catalog no. 65146, Progen Biotechnik, Heidelberg, Germany). We used Progen antibodies to armadillo repeat gene deleted in velo-cardio-facial syndrome (ARVCF) (catalog no. GP155); for its secondary, we used Alexa Fluor555 goat anti-guinea pig (ThermoFisher Scientific, Waltham, MA). We also used a Progen antibody to p0071 (catalog no. 651166) and a Progen antibody for myocardium-enriched zonula occludens-1-associated protein (MYZAP; catalog no. 651169). As a secondary antibody for the DPI/II, p0071, and MYZAP, we used Texas red conjugated goat anti-mouse IgG (ThermoFisher). We classified our findings as negative (−), weakly positive (−/+), positive (+), or strongly positive (+++). We also used an additional antibody to study colocalization in the heart: rabbit anti-connexin 43 (Sigma Aldrich, St. Louis, MO); for its secondary, we used Texas red conjugated goat anti-rabbit IgG.

Colocalization of patient autoantibodies with commercial antibodies using CFM

Our CFM studies were performed as previously described.[7] Standard 20× and 40× objective lenses were used. Each frame included an area 440 × 330 μm. Images were obtained using EZ-1 image analysis software (Nikon, Tokyo, Japan). For colocalization experiments with serum autoantibodies, we used the antibodies to DPI-II, ARVCF, p0071, and MYZAP.

IHC

Our studies were performed as previously described.[7,10] We tested for mouse anti-human IgG, anti-human C3c, α-1-antitrypsin, human matrix metalloproteinase-9, human