Broad histopathologic patterns of non-glabrous skin and glabrous skin from patients with a new variant of endemic pemphigus foliaceus-part 1

A prospective, controlled epidemiologic survey performed in El Bagre, Colombia revealed a new variant of endemic pemphigus disease, occurring in a gold mining region. The disease resembled Senear-Usher syndrome, and occurred in an endemic fashion. The aim of this study is to describe the most frequent histopathologic patterns in non-glabrous skin and in glabrous skin observed in these patients, and their clinical correlation. The study was performed on non-glabrous skin biopsies of 30 patients from the dominantly clinical affected areas (either on the chest, arms or face). Simultaneously, biopsies from the palms were obtained in 10 randomly chosen patients of the 30 total patients. The specimens were examined following hematoxylin and eosin (H&E) staining. The most common blisters observed were subcorneal, although in some cases intraspinous and subepidermal blisters were visualized. Our results showed a very heterogeneous pattern of histopathologic patterns in non-glabrous skin, which seemed to correlate with the clinical features. The most common pattern was typical pemphigus foliaceus-like, with some lupus erythematosus-like features. A non-specific, chronic dermatitis pattern prevailed in the clinically controlled patients taking daily corticosteroids. In the patients who have had the most severe and relapsing pemphigus, early sclerodermatous changes and sclerodermoid alterations prevailed in their reticular dermis. In addition to the sclerodermoid alterations, the reticular dermis showed a paucity of appendageal structures. On the contrary, in the palms, a similar pattern was seen in all cases, including thickening of the stratum corneum, hypergranulosis, edema in the papillary and reticular dermis and a dermal perivascular lymphocytic infiltrate. The direct immunofluorescence of the glabrous vs. the non-glabrous skin also showed some differences. We conclude that the histopathologic features of this new variant of endemic pemphigus are complex, therefore, classical histopathologic features previously described for superficial, endemic pemphigus cannot be used alone to diagnose this disease.
Introduction

Endemic pemphigus foliaceus (EPF) is the only documented, endemic autoimmune-mediated disease observed in a relatively well-defined geographic region.\(^1\)\(^-\)\(^3\) It is generally accepted that pemphigus foliaceus (PF) and Brazilian EPF, also known as fogo selvagem (FS), are characterized by acantholysis and blistering in the upper epidermis, with deposits of predominantly IgG4 autoantibodies in the intercellular spaces.\(^1\)\(^-\)\(^3\) Biopsies from most FS patients with acute disease have shown subcorneal bullae with acantholysis and epidermal clefts.\(^3\)\(^-\)\(^6\) In contrast, chronic lesions from FS patients have been reported to show acanthosis, mild papillomatosis, hyperkeratosis and keratotic plugging of hair follicle.\(^3\)\(^-\)\(^6\) Within the dermis, vasodilatation and infiltration of lymphocytes and histiocytes are often reported.\(^1\)\(^-\)\(^3\)\(^-\)\(^6\) In the group of patients with EPF from Tunisia, the presence of cosinophilic spongiosis with acantholysis was frequently noted histologically, with accompanying clinical features resembling pemphigus herpetiformis.\(^7\)\(^,\)\(^8\)

We have identified a new variant of EPF in the areas around El Bagre, Colombia (El Bagre EPF).\(^9\)\(^,\)\(^10\) Our studies resulted from an 11-year survey, and established that this geographic focus exhibits similar features to those described in pemphigus erythematous, also known as Senear-Usher syndrome, a disease with combined features of lupus erythematosus and pemphigus.\(^11\)\(^,\)\(^12\) El Bagre EPF predominantly affects 40- to 60-year-old males, as well as a few postmenopausal females; the patients are primarily miners who also work in agricultural activities.\(^9\)\(^,\)\(^10\) In contrast, FS primarily affects children and young adults, with the highest incidence at 10- to 30- years of age and both sexes equally affected.\(^1\)\(^-\)\(^3\)\(^,\)\(^9\)\(^,\)\(^10\) The principal aims of our study were to compile and classify the common histopathologic and direct immunofluorescence (DIF) patterns in skin biopsies from affected skin and clinically ‘unaffected’ palms of El Bagre EPF patients, and to correlate these findings with their respective clinical presentations.

Materials and methods

Subjects of study

We studied 30 patients who fulfilled the diagnosis of El Bagre EPF. Our 30 patients were chosen for the study after fulfilling a minimum five out of six of the following inclusion criteria. A clinical stage vs. histopathologic correlation was obtained. Written consents were obtained from all patients, as well as Institutional Review Board permission.

Skin sample

We tested 30 cases of El Bagre EPF previously diagnosed by us that fulfilled the following criteria. Specific criteria include (a) patient displays clinical features described for El Bagre EPF,\(^5\)\(^,\)\(^10\) (b) patient lives in the endemic area, (c) patient serum displays cell surface staining between keratinocytes by either DIF or indirect immunofluorescence (IIF) (specifically, using Fluorescein isothiocyanate (FITC) conjugate monoclonal antibodies to human IgG4 or total IgG in glabrous skin),\(^9\)\(^-\)\(^11\) (d) patient serum immuno-precipitates a Con-A affinity purified bovine tryptic 45-kDa fragment of PF antigen,\(^10\)\(^-\)\(^16\) (e) patient serum is positive by immunoblotting (IB) for reactivity against desmoglein 1 (Dsg1), as well as for plakin molecules as previously described,\(^15\)\(^,\)\(^16\) finally, (f) patient serum is positive using an enzyme-linked immunoabsorbent assay when screening for autoantibodies to PF antigens.\(^16\)

For all the above determinations, serum from a well-characterized FS patient from Brazil and two PF sera from the endemic area were used as positive controls. A negative normal control from USA was also used. Following local anesthesia without epinephrine, skin biopsies were taken from the clinical predominantly lesions from affected areas on the chest, arms or face. Biopsies were fixed in 10% buffered formalin and processed for hematoxylin and eosin (H&E) staining. Biopsies from 10 randomly chosen patients out of 30 total patients were taken from the palms and split into two pieces; one was placed in Michel’s medium for DIF and the other half was fixed in 10% buffered formalin and processed for H&E staining.
DIF and IIF

For IIF, sera from all subjects were titrated in calcium-supplemented buffer at 1 : 20 and 1 : 40 dilutions. We then incubated the substrate tissues with the serum. We next added the secondary antibodies; FITC conjugated rabbit anti-total IgG antiserum (Dako, Carpinteria, California) and FITC conjugated rabbit anti-human IgG4 (gamma chain) (Sigma Aldrich, Saint Louis, Missouri) at dilutions 1 : 20 and 1 : 40, respectively. For IIF, the antigen source was monkey esophagus. The samples were run with positive PF control sera and a negative control serum. For the DIF, we used FITC conjugated rabbit antiserum to human IgG, IgA, IgM, C3, C1q, fibrinogen and albumin. Anti-human IgA antiserum (alpha chain) and anti-human IgM antiserum (mu chain) were obtained from Dako. Anti-human IgE antiserum (epsilon chain) was obtained from Vector Laboratories. The slides were counterstained with 4',6-diamidino-2-phenylindole Dapi (Pierce, Rockford, Illinois). Mouse anti-collagen IV monoclonal antibody (Invitrogen, Carlsbad, California) was used with a secondary donkey anti-mouse IgG (H+L chains) conjugated with Alexa Fluor 555 (Invitrogen).

Immunoblotting (IB)

Sera were tested for reactivity against Dsg1. This was carried out as previously described.9,10,14 We used sodium dodecyl sulfate (SDS) extracts of human and bovine epidermis and fractionated by 7% SDS polyacrylamide gel electrophoresis (SDS-PAGE) according to the Laemmli method.9,10,14 Gels were transferred onto nitrocellulose membranes.5,10

Superimposed microbiological infections

Based on the fact that our patients live in a tropical area in conditions of poverty and malnutrition and given the fact that we had previously detected some superficial subcorneal blisters in several El Bagre EPF patients,9 we felt it necessary to discard samples displaying positive infection with bacteria and fungi. The presence of clinical microbiological infection was assessed by using a smear from the 4-mm skin punch biopsies before fixation in formalin. In addition, culture for fungi and mycobacteria were performed in accordance with the World Health Organization recommendations.17

Results

General histopathologic pattern and correlations with clinical presentations

Based on our experience of working with patients affected by this disease, we were able to observe very broad clinical patterns and histologically heterogeneous lesions. Indeed, approximately 50% of the El Bagre EPF patients had different morphologic clinical lesions at the same time. Superficial erosions with some oozing and marked crusting were noted in some patients. Other patients presented with ulcerated lesions on erythematous skin with weeping yellowish serum surrounded by flaccid, easily ruptured vesicles. In some cases, the lesions were papulovesicles or papulopustules, arranged in arciform, annular or serpinginous patterns. Occasionally the lesions were discrete and localized, especially in more benign cases. Based on established dermatopathologic patterns, we divided our skin findings into several primary histopathologic prototype groups. In about 80% of patients, melanophages were present around the upper dermal vessels.

Typical PF-like histologic features

In about 25% of the cases, the histologic features of PF were observed (Fig. 1A). Clinically, as classically described in PF, the initial bullae are usually present on the trunk. The primary lesions were small superficial blisters (rarely observed clinically); because they are transient and quickly transform into erosions (Fig. 1B). Sometimes the bullae were localized at the basal membrane zone (BMZ) (Fig. 1C).

Lupus erythematosus-like histologic features

This pattern was seen in about 20% cases (Fig. 1D). Clinically, in these cases, discoid patches were clearly observed on the face (Fig. 1E). These cases were therefore clinically almost indistinguishable from classic cases of lupus erythematosus. Some histologic features included basal layer vacuolar change, active interface inflammation, epidermal atrophy, follicular plugging, deep perivascular and periannexal inflammation as well as mucin deposition.

Chronic dermatosis pattern

In about 20% of the chronic cases, in which the patients were taking oral steroids varying between 20 to 40 mg/day and showing improvement in their clinical lesions, the biopsies showed histologic changes resembling those observed in chronic dermatoses (Fig. 1F,G). Clinically, most of these cases displayed lichenification of the skin with small plaques, papules and vesicles, especially in the chest. Occasionally, the only clinical finding was hyperpigmented or erythematous macules, where previous inflammatory lesions had occurred (Fig. 1H). Of interest was the fact that in more senior patients (70 years or older) the chronic lesions became increasingly dry over time, with no vesicles, pustules or weeping. Instead, the main clinical lesions were confluent, hyperpigmented
Fig. 1. Histopathologic patterns and their corresponding clinical lesions from patients affected by this new variant of endemic pemphigus foliaceus (EPF). A) Classical pemphigus foliaceus (PF)-like histopathology is appreciated, with subcorneal acantholysis and an inflammatory infiltrate surrounding the superficial vessels in the dermis. B) A series of bullae on the trunk with denuded areas and few pustules. C and D) Degeneration of the basal layer (C) and the atrophic epidermis (D). E) The face of this patient showed a butterfly rash, a scaling hyperkeratotic plaque on an erythematous base. F and G) Regular, mild acanthosis and focal mild papillomatosis with numerous melanophages are also present within the papillary dermis. H and I) Crusts, hyperpigmented plaques and macules on the shoulder (H) and some papuloplaques around the axilla and torso (I). J and K) A patchy dermal perivascular infiltrate of lymphohistiocytic cells and melanophages is noticed, with some concomitant dilatation of blood vessels. Periapendageal infiltration of lymphohistiocytic cells, including the deep sebaceous glands, resembles focal histopathologic features seen in lupus erythematosus. L) A conjunctival ejection. M and N) A psoriasis-like pattern, with the rete ridges showing regular elongation but with variable thickening of the lower portions, in contradistinction to well-developed psoriasis in which uniform thickening in the lower rete ridges is often noticed. In some EPF cases, the dermal papillae are elongated and edematous, as often observed in psoriasis. In addition, scattered areas of parakeratosis were also seen in these biopsies from patients affected by EPF. O) Well-circumscribed bright red, circular papules and plaques with overlying silvery scale were noted on the trunk, resembling plaque-type psoriasis. P and Q) In contrast to psoriasis, large pustules beneath the cornified layer were common in some EPF patients, overlapping with classic histologic findings of a subcorneal pustular dermatosis. R and S) Note the large pustules on the chests of these patients. T) Axillary plaques of an El Bagre EPF patient, which were a very common clinical finding in this group of patients.
Dry plaques resembling broad seborrheic keratoses. These were mainly distributed under the axillare and sides of the chest (Fig. 1I).

**Polymorphous light eruption-like lesions**

In these cases, histologic characteristics included a superficial and deep dermal infiltrate of lymphocytes, with occasional eosinophils and neutrophils. Early cases might only show changes in the papillary dermis. Edema was often prominent in the upper dermis, and occasionally resulted in subepidermal bulla formation. The epidermis displayed varying degrees of spongiosis, with occasional parakeratosis and acanthosis (Fig. 1J-K). Interestingly, found these lesions typically presented on sun-exposed sites with an erythematous rash composed of micropapules. Several patients also displayed eyelid involvement, which correlates with recent data documenting immunohistologic compromise of eyelids and conjunctiva affected by El Bagre EPF (Abreu et al., manuscript in press in J Am Acad Dermatol) (Fig. 1L).

**Psoriasis-like histologic features**

These occurred in approximately 10% of the cases. Some features included focal epidermal psoriasiform changes, without thickening of the lower portion of the rete ridges (Fig. 1M,N). In a few cases, Kogoj spongiform pustules or Munro-like microabcesses were seen. Clinically, we observed well-demarcated, red-violet, round or circinate plaques in those patients (Fig. 1O). A symmetric distribution of skin lesions was often appreciated in these patients. In contrast to classic psoriasis, the clinical lesions of this group never involved the elbows, extensors surfaces, knees, intergluteal cleft, and genitalia; however lesions, similar to classic psoriasis, were occasionally present on the scalp, sacral and umbilical areas.

**Subcorneal pustular dermatosis-like histologic features**

In about 20% of the cases, histologic features resembled a subcorneal pustular dermatosis with vesiculo-pustules in the epidermis (Fig. 1P). Some microabcesses were seen within the corneal layer, while others were seen in subcorneal or granular cell layers. The subcorneal blisters were mostly filled with fibrinoid material (Fig. 1Q), and occasional erythrocytes without frank hemorrhage were observed. Clinically, these patients presented with pustular, mostly aseptic lesions as demonstrated by the previously described microbiologic studies. However, in contrast to lesions of classic subcorneal pustular dermatosis (Fig. 1R), the flexural aspects of the limbs were also involved except for the axillary regions (Fig. 1S,T).

A large percentage of patients (around 60%) from all El Bagre EPF cases present histologically with a combination of the above-mentioned patterns. For example, among patients with blisters, the most common blisters observed were subcorneal (40%), although in some cases subcorneal, intraspinous and subepidermal were visualized. In some cases, all of these blister patterns were noted simultaneously.

Although superinfected lesions where *Streptococcus* sp and *Staphylococcus* sp were cultured are not included in the current results, clinical lesions in these patients typically presented with ulcerated lesions with large crusts. The clinical presentation of these lesions was a burning sensation and excoriation of the lesions to ameliorate this symptom. Further, the burning sensation *per se* may be due to a healing process (Abreu et al., manuscript in preparation) or, alternatively, may be due to autoreactivity in dermal nerves detected by us in these patients (Abreu et al., manuscript in preparation).

Unfortunately, El Bagre EPF patients displayed no single, common histologic pattern. However, more acute cases have either acantholysis, or clefts or blisters at subcorneal, supraspinous, or BMZ areas. Mild cases and well-controlled chronic cases under prednisone treatment display only mild hyperkeratosis, consistent acanthosis and a large number of melanophages in the superficial dermis. In these chronic patients, the disease manifested clinical hyperpigmented macules and histologically, presence of melanophages surrounding superficial dermal vessels. The most severe cases displayed histologic features including an ulcerated epidermis extending into the deep dermis and involving cutaneous appendices, prominent serum scales, crusts and granulation tissue.

**Palm findings**

Glabrous palm skin showed some dermal inflammation without noticeable acantholysis or BMZ alterations by light microscopy (Fig. 2A,B). A similar pattern seen in all cases, included hyperkeratosis, hypergranulosis, edema in the papillary and reticular dermis (Fig. 2C-G) and some degree of dermal perivascular lymphocytic infiltration (Fig. 2C-E). DIF results for the palm biopsies will be reviewed in a separate publication (Abreu et al., manuscript in preparation).

**IIF using monkey esophagus sections (Fig. 3)**

Anti-IgG antiserum showed a weak epidermal cell surface stain with epidermal granular cytoplasmic pattern. A positivity within vessel walls in the upper dermis was also seen (5/5 cases). When anti-human IgA antiserum was used, 3/5 cases showed positive
Fig. 2. (A) through (F) show hematoxylin and eosin (H&E) staining of biopsies from the palms of ten different patients affected with El Bagre endemic pemphigus foliaceus (EPF); these skin biopsies were taken from the patients simultaneously with other biopsies from non-glabrous skin. A) A small subcorneal separation (black arrow) (200x). B) Mild edema in the papillary dermis and thickened corneal layer on the palmar skin of some patients. Abnormality of the corneal layer, possibly resembling a disorder of keratinization (black arrow). Corneal layer separation may be because of fixation and/or processing artifacts? (yellow arrow) (200x). C) A lymphohistocytic perivascular infiltrate surrounding deep dermal vessels (black arrow) and thickening of the same vessel wall (yellow arrow) (400x). D) A detail of the dermal/epidermal junction area, with some focal areas displaying spongiosis, edema (yellow arrow), lymphocytic infiltration predominantly perivascular (black arrow) and a subcorneal cleavage (blue arrow) (200x). E) Higher magnification (400x). F) A detail of the reticular dermis, with edema (400x). G) In few cases, we observed blister lumina containing erythrocytes but not containing inflammatory white blood cells or fibrin (black arrows) within a vertical column between two histologically adjacent acrosyringia. Stratum corneal keratin within these vertical columns is more transparent compared to adjacent corneal areas (yellow dots). H) Utilizing human epidermal extracts, we performed immunoblotting. We noted the presence of autoantibodies against 300, 250, 230, 190, 200, 160, 117, 97, 55, 45 and 34-kDa molecules in the El Bagre EPF sera (lanes 1–10). Lane 11 is for anti-desmoglein (Dsg1) monoclonal antibody (Progen, Heidelberg, Germany) reactive with the 160-kDa band. Lane 12 is for a negative normal control.

cell surface staining at a 1 : 40 titer. Anti-human fibrinogen antiserum displayed the strongest positivity in these cases, with titers of 1 : 160 in the central lamina propria; the pattern resembled a fine plumage at the BMZ. Collagen IV seemed to colocalize with the immunoreactivity to fibrinogen. When antiserum to IgM and albumin were used, BMZ reactivity was seen, but mostly in epidermal basal cells. IgE was also positive at a 1 : 40 titer, and associated with perinuclear staining around the keratinocytes. Both
Fig. 3. Indirect immunofluorescence (IIF) for El Bagre endemic pemphigus foliaceus (EPF) patients utilizing monkey esophagus as a substrate. A) FITC conjugated anti-human fibrinogen antiserum showed reactivity to the basal membrane zone (BMZ) (green stain) (red arrow). This immunostaining colocalized with monoclonal antibodies to collagen IV (red stain) (white arrow). In addition, the nuclei of the keratinocytes were counterstained with Dapi (blue) (fuchsia arrow) (100x). B) The IIF also showed positive staining with FITC conjugated anti-human IgM antiserum, this staining was manifested as a granular intracytoplasmic staining within the keratinocytes, mostly in the basal membrane areas (green staining) (red arrow). Keratinocyte nuclei were also counterstained with Dapi in this procedure (blue stain) (fuchsia arrow) (400x). C) IIF for (B) was repeated; i.e. FITC conjugated anti-human IgM antiserum showed intracytoplasmic staining in the epithelial keratinocytes (red arrow) and at the BMZ. In this second procedure, colocalization with antibody to collagen IV was also observed (white arrows) (400x). D) Positive deposits of FITC conjugated anti-human IgG antiserum were noted at the BMZ (green stain) (red arrow). In this procedure, the antibody to collagen IV colocalized with the IgG antibody (orange stain) was observed (white arrow). The nuclei of the keratinocytes were again counterstained with Dapi (blue stain) (fuchsia arrow) (600x). F) FITC conjugated anti-human IgE antiserum, showing perinuclear reactivity within the keratinocytes (red arrow) (100x).
Dapi and collagen IV performed well in mapping and colocalization studies.

The IB results

Immunoreactivity to 160kDa Dsg1, in addition to plakins (envoplakin and periplakin), was detected in El Bagre EPF patients (Fig. 2H). Of interest, a band of about 200 kDa and several other bands were also noticed in the sera from the patients with the lupus-like histopathologic pattern that require further scrutiny.

Discussion

The problem in histopathologic differentiation of endemic pemphigus disease is the fact that the current scientific literature regarding FS was generated from the most prevalent clinical form of the disease. Through our 11 years of field work with this El Bagre-EPF, we were able to follow the patients for longer periods. We were thus able to observe document temporal changes in the clinical disease, and show the wide range of histopathologic patterns detected in the patients. Our results show some differences with other types of superficial pemphigus. As in pemphigus erythematosus, the lesions do exhibit histologic features shared with other dermatoses.18,19 The clinical, histopathologic and immunologic data correlate with the wide-ranging number of autoantibodies present in people affected by this variant of EPF. Autoantigens detected in this variant of El Bagre EPF include Dsgs, multiple plakin molecules and other antigens whose nature remains unknown.10 –16 Such a pattern of immunoreactivity may be reflected in the polymorphous clinical patterns detected in the patients affected by El Bagre EPF. One of the largest series of histopathologic studies in patients affected by FS was reported by Furtado in 1959.4 The study consisted of 213 sections from 183 patients of FS. The most common histologic findings were (a) acantholysis (in 91.8% sections), (b) acanthosis (in 74.8%), (c) acantholytic cells (in 68.5%), (d) bulla formation (in 47%), (e) dyskeratosis (in 38.5%), (f) hyperkeratosis (in 31.9%), (g) epidermal appendage changes (in 31.9%), (h) papillomatosis (in 26.3 %) and (i) hyperpigmentation (in 25.5%).4 Other findings such as chronic dermatitis, psoriasiform dermatitis and pustular dermatitis have also been described in patients with FS.12,3 These specific findings are also found within our data. Larger comparative studies might yield insights by simultaneously correlating clinical, histologic and immunologic findings. Of interest was the presence of the melanophage-like cells in the skin of these patients; this finding seems to arise not solely because of a passive pigment transfer process. We base our suggestion on three findings: (a) the presence of patchy, liquefac-tive areas at the basement membrane zone detected by H&E staining; (b) focal immunoreactivity at the basement membrane zone identified by DIF and IIF and (c) the presence of multiple melanophages and dendritic-like cells in these areas. These cells probably display an active pigment scavenging function; they may provide a barrier against ionizing radiation, and also perform as scavengers of cytotoxic free radicals and intermediate molecules.

Of note is the fact that patients taking prednisone 20–40 mg/day when the biopsies were performed showed some of the atypical histologic features (i.e. different from the usual PF pattern). Those biopsies showed papillomatosis and hyperkeratosis and a large number of melanophages. Our observation is of importance, since such medication can in fact affect the pattern appreciated on histologic review.

Two important findings were the detection of inflammation in the palms and mild acantholysis in these biopsies. Also, frank separation at the granular layer was not seen in the palms, in contradistinction to non-glabrous skin areas; in the palms, cleavage was found in subcorneal areas. As the corneal layer is thickened in the palms, we cannot determine based on our current data whether this finding was actually part of the disease process in the palms or was due to an artefact in the biopsy fixation process. Of additional interest is the issue of whether sebaceous glands play an active role in the intensity of this disease on non-glabrous skin, since all the other epidermal appendages are present in the glabrous skin, with exception of these glands. Of importance is the fact that in the chronic stages of the disease, the presence of histologic sclerodermoid changes, lipodermatosclerosis and periappendageal inflammation may cause the loss of appendages in patients affected by El Bagre EPF.

In conclusion, our findings indicate that the pathophysiology of El Bagre EPF represents a dynamic, largely undefined process, with possible overlapping features of other autoimmune molecular processes. We suggest that the diagnosis of EPF should not be based solely on histopathologic findings, but also be accompanied by clinical, epidemiologic and immunologic criteria.

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References