Palm tissue displaying a polyclonal autoimmune response in patients affected by a new variant of endemic pemphigus foliaceus in Colombia, South America

We previously described a new variant of endemic pemphigus foliaceus in El Bagre, Colombia, South America (El Bagre-EPF). On physical examination, the palms and soles of El Bagre-EPF patients reveal an edematous texture and mild hyperkeratosis, in comparison with the non-glabrous skin of the patients where blisters, pustules or other lesions are commonly found. Based on the preceding observation, we tested the palms of 20 El Bagre-EPF cases and 20 controls from the endemic area for any pathological alterations in the samples by direct immunofluorescence (DIF). Our DIF demonstrated pathological deposits of fibrinogen and albumin, as well as IgG, IgA, IgM, IgD and C3c, at 1) the epidermal basement membrane zone; 2) around isolated areas in the epidermis, 3) within the dermal vessels and nerves, and 4) in areas surrounding dermal neurovascular structures and sweat glands. Specific markers for blood vessels, including 1) anti-intercellular adhesion molecule 1 (ICAM-1)/CD54, and 2) anti-junctional adhesion molecule (JAM-A); as well as specific markers for nerves, including 1) anti-glial fibrillary acidic protein (GFAP), and 2) anti-human neuron specific enolase (NSE) co-localized with the patients’ autoantibodies. Although no blisters, ulcerations, pustules or erosions are clinically observed on the palms of El Bagre-EPF patients, our DIF detected distinct immunoreactivity in palm tissue. These alterations may contribute to the clinically edematous texture of the palms and the mild clinical hyperkeratosis found in most of these patients. We propose that normal glabrous skin and non-glabrous skin may be different with regard to the expression of selected molecules, which may vary in number, size or structural organization depending on their anatomical site. Our findings may also partially explain the hyperkeratotic palms that have been clinically well documented in the chronic phase of fogo selvagem i.e., endemic pemphigus foliaceus, in Brazil.

Key words: palms, pemphigus, autoantibodies, nerves, vessels
exfoliation on the palms and soles [7]. The cause of this disease phenomenon is not yet well characterized, although several interesting studies and theories have been described. Moreover, glabrous skin, non-glabrous skin and mucosa seem to be constitutionally different in regards to desmosomes and other types of cell junctions [7]. Thus, based on our observation that El Bagre-EPF patients present pathologically edematous skin on the soles and palms with no apparent blisters, vesicles or erosions, we searched for any subclinical, pathophysiological changes in the palms of El Bagre EPF patients by direct immunofluorescence (DIF).

Materials and methods

Subjects

We studied 20 patients who fulfilled the criteria of El Bagre-EPF, as described previously, and who were followed by us for over a decade [2]. Patient consents were obtained with Institutional Review Board permission. Using classical indirect immunofluorescence (IIF) as described by Beutner et al. [9], patients were considered positive if they had intercellular staining between epidermal keratinocytes, especially with anti-human IgG4 monoclonal antibodies, as well as positive basement membrane zone (BMZ) staining in some cases [1, 2]. In addition to these criteria, the patients lived in the endemic area, and their sera immunoprecipitated a 45 kDa bovine tryptic fragment of the ectodomain of DesG1 [2]. Sera from all patients and controls were also tested by immunoblotting (IB) using human epidermal extracts, as previously described [1, 2]. For all of our above determinations, serum from a well-characterized PF patient located outside the El Bagre EPF endemic area was used as a positive control. In brief, following local anesthesia without epinephrine, skin biopsies were taken from clinically unaffected palms and kept either in 10% buffered formalin (for H & E examination) or in Michel’s transport medium (Newcomer Supply, Middleton, Wisconsin, USA) for DIF. The soles were not biopsied as many of our patients had intercellular staining between epidermal keratinocytes, especially with anti-human IgG4 monoclonal antibodies, as well as positive basement membrane zone (BMZ) staining in some cases [1, 2]. In addition to these criteria, the patients lived in the endemic area, and their sera immunoprecipitated a 45 kDa bovine tryptic fragment of the ectodomain of DesG1 [2]. Sera from all patients and controls were also tested by immunoblotting (IB) using human epidermal extracts, as previously described [1, 2]. For all of our above determinations, serum from a well-characterized PF patient located outside the El Bagre EPF endemic area was used as a positive control. In brief, following local anesthesia without epinephrine, skin biopsies were taken from clinically unaffected palms and kept either in 10% buffered formalin (for H & E examination) or in Michel’s transport medium (Newcomer Supply, Middleton, Wisconsin, USA) for DIF.

Direct immunofluorescence (DIF)

For DIF, four um thick skin cryosections were partially fixed with paraformaldehyde, rinsed in phosphate-buffered saline (PBS, pH 6.8) incubated with fluorescein isothiocyanate (FITC) in a solubilization buffer (PBS with 0.5% Triton X-100 Octylphenolpoly (ethylene glycolether)), and then rinsed. After blocking with PBS with 0.01%-Tween 20 (Polysorbate 20) and 0.5% bovine serum albumin (BSA), the sections were incubated with antisera for one hour. We used FITC-conjugated rabbit antisera against fibrinogen and albumin at 1:40 dilution. These antisera, as well as FITC-conjugated rabbit anti-human IgG (gamma chain) and IgA (alpha chain) antisera (both 1:20 dilution) and FITC-conjugated rabbit anti-human IgM (Mu-chain) antisera were purchased from Dako (Carpinteria, California, USA). Goat anti-human IgE (Epsilon-chain) antisera, conjugated with FITC, was obtained from Vector Laboratories, Inc., Burlingame, California, USA. Goat antiserum FITC conjugated to human C1q and human IgD was obtained from Southern Biotech (Birmingham, Alabama, USA). Anti-human IgD antiserum was absorbed with human IgG, IgM, and IgA. In addition, we used anti-intercellular adhesion molecule 1 (ICAM-1/CD54) antibody (Lab Vision Corporation, Thermo Fisher, Fremont, California, USA) and anti-junctional adhesion molecule (JAM-A) antibody (Invitrogen, Carlsbad, California, USA) (recognizing type I transmembrane glycoproteins of the immunoglobulin superfamily that are localized in the tight junctions). The slides were then counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (Pierce, Rockford, Illinois, USA), washed, coverslipped and dried overnight at 4 °C. Other antibodies used included secondary donkey anti-mouse IgG heavy and light chains (H + L) antiserum conjugated with Alexa Fluor® 555 (Invitrogen) for ICAM-1/CD54, utilized to determine potential co-localization of patient autoantibodies to vessels. In order to determine co-localization between nerves and El Bagre EPF patient autoantibodies, we also used simultaneous staining with monoclonal anti-gial fibrillary acidic protein (GFAP) (Clone GA-5, Cy3-conjugated, Sigma Aldrich, Saint Louis, Missouri, USA), at a dilution of 1:150. We also used mouse anti-human neuron specific enolase (NSE) monoclonal antibody (at 1:40 dilution, also from Dako), and, as a secondary antibody, we utilized Texas red-conjugated sheep anti-mouse IgG (H & L) antiserum (Rockland Immunochemicals, Inc., Gilbertsville, Pennsylvania, USA) at 1:100 dilution. Finally, the sections were examined with a Nikon Eclipse 50i microscope (Tokyo, Japan) using a Xenon arc light (XBO 75W) as the light source and a plane achromatic (PL Apo) × 40/0.80 dry objective. The fluorescent staining was graded as follows: – (negative), + (doubtful), + (weak), ++ (moderate), and +++ (bright). The slides were then examined using Nikon triple filters, i.e., DAPI/FITC/Texas Red (EX 395-410/490-505/560-585 nm, EM 450-490/515-545/600-652 nm).

Statistical analysis

A paired-sample t-test was used to analyze the data, with MedCal software Version 9.6.4.0-©1993-2008, Broekstraat 52, 9030 (Mariakerke, Belgium). Standard deviation with a 95% confidence interval (CI) was determined.

Results

Hematoxylin and eosin findings on palms

Similar patterns were found in most of the cases of El Bagre-EPF. The most common findings included epidermal spongiosis, variable necrotic keratinocytes along the BMZ, and a mild, superficial, perivascular dermal infiltrate of lymphocytes and histiocytes. Melanin pigment dropout was noted in the superficial papillary dermis in most patients. In addition, we consistently observed a mildly hyperkeratotic epidermal stratum corneum; moderate, diffuse edema in both the papillary and reticular dermis, and variable degrees of deep dermal perivascular, perineural, and perineural sweat gland infiltration by lymphocytes and histiocytes.
Direct immunofluorescence studies on palms

No controls from the endemic area were positive by DIF, with two exceptions that each showed some intracytoplasmic staining in patchy areas of the epidermal keratinocytes using C1q. (++). We characterized the positive patterns of DIF as follows: 1) DIF cell surface, or intercellular cell staining between epidermal keratinocytes (ICS). The ICS pattern was noted when using anti-human IgE, IgG, IgM, C3c, albumin and fibrinogen, and in some cases with IgA and IgD. 2) Basement membrane zone (BMZ) staining, in a linear, a lupus-band like pattern. The BMZ linear pattern was primarily observed when utilizing C3 or C3d, IgM, albumin and fibrinogen. 3) Positive staining of the sweat glands, including the gland coils and the acrosyringia, was found with IgE, IgM, IgG, and especially strongly at the acrosyringium with C1q. 4) Positive staining on neurovascular units, especially within nerve structures per se, and occasionally within the perineurium, epineurium, and/or endoneurium areas, as well as within some mechanoreceptors. The neurovascular staining was seen primarily with IgM, C3c, IgA, albumin and fibrinogen. 5) Within the epidermal keratinocytes, positive staining was observed in perinuclear, cytoplasmic and/or peri-plasma membrane locations; this staining was noted with albumin, C1q, IgM, albumin and fibrinogen. 6) A strong immunoreactivity was observed to the superficial, intermediate and the deep dermal blood vessels. This positivity was observed using IgG, IgA, C1q, IgM, albumin and fibrinogen. 7) A dermal smooth muscle a) granular intra-myocyte pattern, and b) intercellular-junction-like pattern was seen, especially with IgG, albumin and fibrinogen (figures 1 and 2). As shown in table 1, the most common patterns were those of reactivity to the sweat glands, to the dermal blood vessels, to the BMZ and, to a lesser extent, to the epidermal keratinocyte intercellular surfaces (ICS). Of interest is the fact that the ICS was often inconsistently distributed throughout the epidermis, varying with the immunoglobulin tested.

Polyclonality of the immune response in palms by DIF

Of particular interest was the polyclonality of the immune response. Specifically, a consistent band-like staining was clearly visualized, especially when using anti-albumin and anti-fibrinogen anti-sera. The bands resembled those seen in lichen planus, but instead of being located within the dermis, the bands were observed throughout the epidermis. The bands seemed to extend from the BMZ upward into the epidermal stratum spinosum. In figure 1, we show several representative pictures of this phenomenon. Figure 1A shows a clinical photograph of an El-Bagre EPF patient. Figures 1B-I shows immunostaining observed in the palms of El Bagre EPF patients i.e., shaggy deposition of fibrinogen at the BMZ and cytoplasmic staining in keratinocytes for albumin and C3. These patterns seemed to form a complex net pattern that intersects or overlaps at some axis points, like some types of cell junctions (figure 1G).

In figures 2A to E, we show representative positive DIF staining for fibrinogen (A and B), albumin (C and D) and C3 (E), displaying a funnel-shaped staining in the acrosyringium and BMZ of sweat glands (red arrows). In figures 2F, G, H and I, we show round, positive IgD staining structures close to the blood vessels. These vesicle-like structures were visualized under 1000x magnification (red arrows). In figure 2H, the yellow arrow displays positive co-localization with the blood vessels, as indicated by positive JAM-A staining (in red). These findings suggest disease autoreactivity to blood vessels in El Bagre-EPF.

Figure 3 also summarizes representative patterns of staining detected in blood vessels in the superficial and deep dermis, as well as in dermal muscle and nerve bundles. We detected co-localization of blood vessel or nerve markers with El Bagre-EPF disease auto-antibodies, indicating that these structures may play a role in the pathophysiology of the disease. In the DIF results, we found a statistically significant difference in the disease cases versus the controls (p > 0.05). In several patients, we detected intracytoplasmic autoreactivity to myocytes, especially for IgM. In agreement with the DIF findings, we visualized H&E lymphohistiocytic inflammation around the dermal nerve bundles, blood vessels and sweat glands. In figure 3I, we detected co-localization of the El Bagre EPF disease antibodies to fibrinogen, albumin and C3 with neurovascular structures exhibiting positive staining with NSE.

Discussion

Hereditary skin disorders caused by desmosomal gene pathology may preferentially involve the palms and soles [6-8]. Indeed, even in normal control skin, it has been previously demonstrated that differences in desmosome number, size and/or structural organization may exist in palmar plantar sites compared to skin from other body regions [6-8]. Further, confocal microscopy of skin biopsy material from glabrous and non-glabrous skin has shown relative differences in the expression profiles of several desmosomal proteins (i.e., desmogleins, desmocollins, desmoplakin, plakoglobin and plakophilin), between these sites. Specifically, a higher expression level of all five of these proteins, as well as a respective early expression of involucrin has been demonstrated in palm skin compared with cultured breast skin keratinocytes. Morphometric analysis has also shown that, overall, desmosomes are larger but of similar population density in the palm compared with breast skin [8]. Although our study results demonstrate no microscopic blisters in the palms or soles of the El Bagre-EPF patients, our DIF findings suggest that immunological events in the palms may be causally associated with the clinical feature of palm edema in this disorder, and possibly mild palmar hyperkeratosis as well. Relative to previous immunodermatology studies, we visualized many similar DIF patterns in our findings, such as ICS and BMZ staining [9, 10]. However, in addition to these patterns, we observed immunoreactivity to sweat glands, blood vessels, neural structures, and both intracytoplasmic and intranuclear staining within epidermal keratinocytes. For over thirty years, immunodermatopathology laboratories have predominately analyzed autoantibodies to IgG, IgM, IgA, C3 and fibrinogen in the evaluation of pemphigus and bullous pemphigoid, following the pioneering work of Beutner, Jordon, Chorzelski and others [9, 10]. Notably, albumin, fibrin and fibrinogen represent consistent immunoreactants in
most autoimmune dermatoses, as well as other inflammatory dermatoses. However, the specific pathophysiological roles of these proteins have not been extensively evaluated in many disease processes. Histologically, skin edema and inflammation are usually associated with increased porosity and fluid leakage in selected basement membrane zones [11]. These changes in BMZ barriers enhance the capacity of fluids and molecules to pass through the BMZ, and move into affected tissues [11]. During the inflammatory process, both fibrinogen and fibronectin are deposited in BMZs; their roles in the leakage phenomenon had previously been interpreted as part of a healing process [11]. It is also known that chronic inflammation in a particular tissue is usually accompanied by a thickening of the BMZ [11]. As the plasma proteins albumin and fibrinogen pass through the BMZ in large amounts, portions of these proteins are retained at the BMZ [11]. The retention of these proteins at the BMZ is well documented in lupus erythematosus [12]. Fibrinogen is an integral component of a primary plasma proteolytic system involved in coagulation and tissue fluid homeostasis. Albumin plays sentinel roles in the maintenance of blood and tissue osmotic pressure, and blood viscosity [11]. In pemphigus and pemphigoid, and

Figure 1. Positivity by DIF in the palm epidermis of patients affected by El Bagre-EPF. A) Shows positive deposits of fibrinogen at the BMZ in a shaggy pattern in the palm from one El Bagre-EPF patient (yellow arrow), in contrast to the plaques seen on his chest (red arrow). B) and C), Show positive deposits of fibrinogen at the BMZ in a shaggy pattern (red arrows). D) Deposits of albumin are observed at the BMZ in a linear pattern (red arrow). E) A band-like staining for albumin, evenly distributed in the epidermis where initial keratinization occurs (red arrows). F) Large, dotted cytoplasmic staining in the keratinocytes for albumin (green stain) (red arrow). The nuclei were counterstained with DAPI (blue). G) Similar to F), but, in this case, the red arrow points out positive staining for C3. The yellow arrow shows simultaneous deposition of C3 in a linear manner at the BMZ. H) Positive staining for FITC conjugated IgG to mechanoreceptor (red arrow), which shows positive co-localization of the El Bagre-EPF antibodies, and GFAP (orange staining) (red arrow). I) The nuclei were counterstained in light blue with DAPI.
possibly in many other autoimmune skin diseases, at least one of the four primary plasma proteolytic systems appears to be consistently affected. The four systems are: 1) the complement system (e.g., C1q, C3, and C4 [13-15], 2) the kinin system (e.g., kallikrein), 3) the blood coagulation system (e.g., fibrinogen), and 4) the blood fibrinolytic system (e.g., urokinase-type plasminogen activator and plasminogen) [13-15]. Albumin serves as the primary carrier of many of these pathway molecules, and may also perform a secondary transport function when the primary carriers are not available [11].

Based on our findings, as well as the current medical literature, we speculate that both fibrinogen and albumin may play more than a passive role in the pathophysiology of autoimmune and other inflammatory skin disorders. In animals, the immunological role of these proteins may be multifaceted (i.e., as hapten carriers, co-stimulatory molecules, and/or other functions) [16]. For example, in the equine disease strangles, caused by Streptococcus equi, S. equi M protein (SeM3), is also known as fibrinogen binding protein (FgBP) and serves as a major cell wall-associated protein of S. equi subsp. Equi. The N-terminal region of FgBP can bind strongly to fibrinogen and thus appears to play an important part in the anti-phagocytic effect of the protein, and survival of S. equi in the host. In addition to the binding of host fibrinogen, FgBP has been demonstrated to bind to the Fc region of equine IgG, as well as IgG from several other species [16].

**Figure 2.** Depicts DIF positivity in some palm appendageal structures, and around dermal blood vessels. A) Fibrinogen (A and B), albumin (C and D) and C3 (E) showing funnel-shaped staining on the acrosyringium of the sweat gland, and on the adjacent BMZ (red arrows). A) and B) The nuclei were counterstained with DAPI (blue). E) Similar to D) but the nuclei were stained with DAPI (red arrows). We found round structures close to the vessels that stained positive exclusively with human IgD antiserum (F, G, H, and I). F) These vesicles were attached to one vessel (400×). G) and I) 1000× (red arrows). H) The yellow arrow shows close proximity of the round structures to the vessels stained by JAM-A (in red).
In studies of human cell interactions with extracellular matrices, studies have shown that albumin and other molecules can alter the matrix biochemical properties, which may affect the growth and/or morphologic properties of the target cells [17]. We suggest the possibility of a currently uncharacterized, fluid-based albumin and/or fibrinogen matrix that may allow these proteins to play such a role in vivo in multiple dermatoses, thus facilitating the homing of host inflammatory cells into the target tissues [18, 19].

In our study, we found auto-antibodies from El Bagre-EPF patient sera directed to the dermal blood vessels (both papillary and reticular), which co-localized with ICAM-1/CD54 and JAM-A. ICAM-1/CD54 is present in endothelial cells, and mediates cell adhesion by binding to Integrin CD11a/CD18 enhancing antigen-specific T-cell activation. ICAM-1/CD54 also binds to CD43 and to CD11a/CD18 enhancing antigen-specific T-cell activation. Notably, clinical palmar involvement has been described in patients with pemphigus vulgaris [24, 26]. Palmar immunobullous disease and acquired PKP have also been reported in a patient with alterations in desmocollin 3 proteins [25].

Positive IgD deposits were found in a few palms of our El Bagre-EPF patients. IgD deposits have previously been described in several cases of pemphigus and BP [27]. Deposits of IgD have also been described in patients with FS [28]. Of importance is the fact that the EPF foci occur in areas exposed to multiple tropical diseases, including malaria, leishmaniasis, hydatidosis, scutiosomiasis, and trypanosomiasis. Thus, the observed immune response to IgD might be secondary to concurrent, separate disease processes, and may or not play a pathogenic role in El Bagre EPF [2, 3].

Similarly to our findings, the presence of auto-antibodies to smooth muscle has been reported in paraneoplastic pemphigus and in pemphigus associated with myasthenia gravis [29, 30]. In addition, and similarly to our findings, smooth muscle reactivity within blood vessels has been previously documented in autoimmune skin disorders [31, 32]. In patients with chronic fogo selvagem, the presence of palmpoplantar hyperkeratosis has been also described [28].

In summary, we present DIF immunological findings that may contribute to the pathophysiology of the clinical palmar edematous and mild hyperkeratosis observed in patients affected by El Bagre-EPF. The full significance of our findings is unknown, and warrants further investigation. Our findings do not address the clinical lack of palmpoplantar blisters, vesicles and pustules in the El Bagre-EPF patients, in comparison with other areas of their skin. We speculate that hair follicles and sebaceous glands (not present in the palms and soles) may play an

Table 1. The most common patterns of detection of autoantibodies in the case El Bagre EPF cases versus the controls

<table>
<thead>
<tr>
<th>Immunological findings by DIF</th>
<th>El Bagre-EPF</th>
<th>Controls endemic area</th>
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<tbody>
<tr>
<td>DIF Intercellular cell keratinocyte surface (ICS)</td>
<td>IgG (+), (70%), C3 and rare IgA and IgM, albumin (+) (70%)</td>
<td>Negative</td>
</tr>
<tr>
<td>DIF BMZ (Lupus-band like)</td>
<td>IgG (+) (58%), albumin (+) (85%), fibrinogen (++) (95%), IgM (+) (40%), IgA (++) (34%).</td>
<td>Negative</td>
</tr>
<tr>
<td>DIF sweat glands acrosyringium</td>
<td>IgG (+) (38%), albumin (+) (38%), fibrinogen (++) (40%), IgM (++) (20%), IgE, C3, C1q (++) (20%)</td>
<td>Albumin (+) (15%), fibrinogen (+/-) (15%)</td>
</tr>
<tr>
<td>DIF Sweat glands coiled portion</td>
<td>IgG (+) (38%), albumin (+) (38%), fibrinogen (++) (40%), C3 (++) (345)</td>
<td>Negative</td>
</tr>
<tr>
<td>DIF nerves bundles and or mechanoreceptors (Abreu et al., ms submitted)</td>
<td>IgA (++) (34%), ICAM-1 (++) (43%), IgM (++) (34%), albumin (++) (35%), fibrinogen (++) (35%),</td>
<td>Negative</td>
</tr>
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</table>
| Pery-nuclear, some cytoplasmic and/or periplasma membrane. This was mostly granular. | Albumin (++) (70%), IgM (++) (30%), IgG (++) (50%). | Albumin (+/-) (13%), IgM (+/-) (13%), fibrinogen (+) (15%)
| Superficial and deep vessels | Fibrinogen (++) (90%), ICAM-1 (++) (73%), IgA (++) (28%), IgM (++), C3 (++) (30%), IgG (++) (50%), JAM-A (25%), IgE, C3, C1q (+) (20%). | ICAM-1 (+/-) or (--) 100%, IgD (20%)
| Deep muscle granular intra-myocyte patterns | IgA (++) (28%), IgM (++) (30%), fibrinogen (++) (30%), albumin (++) (50%), IgG (++) 35% | Negative Fibrinogen (+/-) (10%), (+/-) (10%), albumin |
| Deep muscle intercellular-like junction pattern | IgA (++) (28%), IgM(++) (30%), fibrinogen (++) (30%), albumin (++) (50%), IgG (++) 35% | Negative Fibrinogen (+/-) (10%), (+/-) (10%), albumin |
Figure 3. DIF in the palms of El Bagre-EPF patients shows autoreactivity to dermal blood vessel, muscle and nerve receptors. 
A) In a deep palm biopsy, a positive staining, yellowish structure with a “net” shape was seen when using anti-fibrinogen antibodies (yellow arrow).  
B) When utilizing JAM-A antibody (red staining), we were able to demonstrate co-localization with the patient autoantibodies (1000×). The nuclei were counterstained with DAPI (blue).  
C) IgG reactivity to dermal blood vessel areas (green arrow); positive staining in the same area was demonstrated by ICAM-1/CD54. The co-localization of both stains (IgG/green and ICAM-1/CD54/red) resulted in a prominent orange staining effect.  
D) IgG reactivity to other deep vessels (green stain) (red arrow).  
E) A schematic representation of the skin (with permission for reproduction from the Skincare Forum, Issue 29) showing a porus sudoriferus.  
F) Positive cytoplasmic myocyte staining for IgM (dotted yellowish staining) (red arrow). The nuclei of the myocytes were counterstained with DAPI (blue).  
G, I, J, K) (DIF) and H) (H & E) showing reactivity to nerves and nerve-related structures.  
G) Positive staining to nerves using anti-human IgM and IgA antisera (red arrow).  
H) H & E, demonstrating lymphohistiocytic cells around the nerve bundles (black arrows).  
I) IgG reactivity to possible Krause end bulbs (green) (yellow arrow). The nuclei were stained with DAPI (blue).  
J) Strong green staining for IgM and IgG (resembling background in the dermis) (red arrow); however, when evaluating colocalization of this “background” dermal reactivity with NSE (pink) (yellow arrow), both IgM and IgG colocalized.  
L) IgG reactivity to small dermal blood vessels in the palms, (green staining) (red arrow); JAM-A staining was also performed (red stain) (white arrow).
essential role in blister formation in the non-glabrous skin of these patients. We further suggest that the specific qualities of the glabrous skin epidermis (i.e., its increased stratum corneum thickness, potentially thicker keratinocyte cell membranes within the stratum spinosum, and the additional epidermal stratum lucidum) may play a critical role in protection against external trauma and that such traumatic forces may directly contribute to skin vesicle and bulla formation in patients affected by this disorder.

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