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DERMATITIS HERPETIFORMIS BODIES AND AUTOANTIBODIES TO NONCUTANEOUS ORGANS AND MITOCHONDRIA IN DERMATITIS HERPETIFORMIS

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Abstract
Introduction: The precise nature of the previously described dermatitis herpetiformis bodies remains unknown.
Aims: Our study was conducted to investigate the nature of dermatitis herpetiformis bodies in the skin in 7 cases of dermatitis herpetiformis, and to search for the presence of autoantibodies in other organs.
Methods: We utilized clinical, histopathologic, and immunologic methods to evaluate these patients.
Results: Dermatitis herpetiformis bodies were found to be comprised of an amalgamation of immunoglobulins A and M, as well as molecules reactive with antibodies to armadillo repeat gene deleted in velo-cardio-facial syndrome, desmoplakins 1 and 2, and plakophilin 4. In addition, we found immunologic colocalization with selected autoantibodies associated with mitochondria in the skin, heart, kidney, and peripheral nerves. The dermatitis herpetiformis bodies did not demonstrate immunologic colocalization with tissue/epidermal transglutaminase.
Conclusions: The complete biochemical nature of dermatitis herpetiformis bodies requires further characterization. Dermatitis herpetiformis bodies in these patients appear to be distinctly different than cytoid bodies. Further studies are required to determine if the antibodies to noncutaneous organs are pathogenic, and/or contribute to systemic morbility in dermatitis herpetiformis patients.

Key words: dermatitis herpetiformis; endomysium; mitochondria; plakophilins; p120 ctn molecules; desmoplakins

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Introduction

Dermatitis herpetiformis (DH), previously known as hydroa or Duhring-Brock disease is an autoimmune, subepidermal blistering disease characterized by IgA deposits in the dermal papillae [1-2]. DH predilects females and Caucasians, and is a rare disease. The eye and oral cavity are very seldom affected. Immunoglobulin, complement and epidermal transglutaminase (eTG) deposition has been previously detected in the cutaneous blood vessels in dermatitis herpetiformis [3]. A gluten dependent enteropathy may accompany DH.

The mucosa of the small intestine is often atrophic in patients with DH, and autoantibodies to gliadin and endomysial antigens have been found in most patients, as well as antibodies to tissue transglutaminase (tTG) [3]. Moreover, DH is also associated against eTG; eTG represents a member of the same protein family as tTG. Further, tissue transglutaminase has been described as the primary autoantigen of celiac disease [3]. However, one study of 132 patients with celiac disease showed that only 15 of them were associated with dermatitis herpetiformis [4]. In another study of 22 patients with dermatitis herpetiformis, the authors searched for IgA antibodies against endomysial antigen (EmA) and tTG and only found these antibodies in 50% and 62.5% of their sera, respectively. Small intestinal biopsies confirmed the presence of celiac disease in only two patients [5].

Pruritus is the predominant DH symptom; the eruption is symmetric, with a predilection for the shoulders, buttocks, elbows, and thighs and extensor areas [1].

The most common dermatologic findings in DH are 1) weals, papules, vesicles or bullae (sometimes grouped), 2) scratch marks (often complicated by secondary pyogenic infections), 3) pigmented macules, and 4) occasional scars following primary lesion healing. The etiology of the disease remains obscure; sulfapyridine and dapsone are therapeutic drugs of choice [1]. The eruption will reappear promptly following complete suppression if therapy is discontinued.

Besides the granular and/or fibrillar deposits of IgA in the dermal papillary tips, the presence of dermatitis herpetiformis bodies (DHBs) in the papillary dermis of affected skin has been demonstrated; their nature remains unknown [6]. Previous studies using Gold conjugated immuno-electron microscopy have demonstrated the DHBs to be IgA positive, and identified amorphous clumps interpreted as immunocomplex aggregates scattered throughout the upper papillary dermis [7]. Dermatitis herpetiformis bodies were seen underneath the basement membrane zone (BMZ), sometimes along microfibrillary bundles, as well as adjacent to papillary collagen fibers and within the microfibrillar region of elastic tissue. Some dermatitis herpetiformis bodies, however, were described as not associated with any fibrillar components. Notably, the collagen and elastic fibers, microfibrillar bundles, anchoring fibrils, and elastic microfibrils per se were unlabelled. In our cases we searched for DH autoantibodies in the skin, in the dermatitis herpetiformis bodies and in other organs to further elucidate their nature.

Material and Methods

We studied 7 cases of DH whose diagnosis was established based on history, clinical and histological features, and immunological tests [1,2]. Before entering the study, all the patients and controls gave written informed consent; the study was approved by the local Ethics Committee. In all patients of the study, histologic examination revealed neutrophilic infiltrates and neutrophilic abscesses; in selected cases, subepidermal blisters were also noted. Classic papillary microabscesses were observed in 5 biopsies. Patients in the study demonstrated by direct immunofluorescence (DIF) the presence of granular deposits of IgA in skin dermal papillae. Indirect immunofluorescence testing was positive for IgA endomysium antibodies (Esophagus monkey IgA EmA, Medizinische Labordiagnostica, Denmark), in 6/7 patients (titer 1:10–1:320, median 1:40). Anti-tissue transglutaminase antibodies were measured using ELISA (Celikey, Pharmacia & Upjohn, Freiburg, Germany), and were present in 4 out of 7 cases (median 4.3 IU/mL). For each patient, we performed two lesional skin biopsies from clinical blisters. The first biopsy was fixed in 10% buffered formalin and submitted for hematoxylin and eosin (H&E) staining, as well as immunohistochemistry (IHC). The second biopsy was placed in Michel's transport medium, and submitted for DIF. Serum was obtained for IIF studies. Our DIF, IIF and IHC studies were performed as previously described [7-13]. As controls, we tested skin from seven healthy patients undergoing breast and/or abdominal aesthetic reduction surgeries. Biliary cirrhosis was ruled out in all the subjects of the study.

Colocalization of dermatitis herpetiformis bodies with known antibodies utilizing confocal microscopy:

We utilized standard 20 and 40X objective lenses; each photoframe included an area of approximately 440 x 330 μm. Images were obtained using EZ-1 image analysis software (Nikon, Japan). For colocalization experiments with the dermatitis herpetiformis serum autoantibodies, we used antibodies to desmoplakins 1 and 2, (DI-DPII), armadillo repeat gene deleted in velocardiofacial syndrome (ARVCF) and anti-p0071 (Progen Biotechnik, Heidelberg, Germany). Our studies were performed as previously described [7-13].

Immunoblotting (IB):

IB testing was performed as previously described [12]. In brief, we used sodium dodecyl sulfate (SDS) extracts of human and bovine epidermis 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). As experimental controls, we utilized 1) an anti-desmoplakin multiepitope cocktail from Progen (Heidelberg, Germany), 2) the serum of a patient with bullous pemphigoid that recognized bullous pemphigoid antigens 1 and 2 (230 and 180 kDa, respectively), and 3) serum from a patient with paraneoplastic pemphigus. Our IB was performed as previously described [7-13].

Direct and indirect immunofluorescence (DIF and IIF):

For DIF and IIF, sera from all subjects were titrated in PBS IX buffer at 1:25 and 1:40 dilutions. We then incubated the substrate tissues with the serum. We next added the secondary antibodies; we utilized FITC conjugated rabbit anti-total IgG (Dako, Carpinteria, California, USA) and FITC conjugated rabbit anti-human IgG4 (gamma chain; Sigma Aldrich, Saint Louis, Missouri, USA) at dilutions 1: 20 and 1:40, respectively. For IIF, the antigen source was monkey esophagus (ME). The samples were run with positive DH control sera and a negative control serum. For the DIF, we used FITC conjugated rabbit antisera to human IgG, IgA, IgM, C1q, C3, 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 (Burlingame, California, USA). Anti-human IgD FITC-conjugated antibodies were also used (Southern Biotechnology, Birmingham, Alabama, USA).
The slides were counterstained with 4',6-diamidino-2-phenylindole Dapi (Pierce, Rockford, Illinois, USA). Mouse anti-collagen IV monoclonal antibody (Invitrogen, Carlsbad, California, USA) was used with a secondary donkey anti-mouse IgG (heavy and light chains) conjugated with Alexa Fluor 555 (Invitrogen).

Immunohistochemistry (IHC):
We performed IHC to differentiate between specific autoreactivity and non-specific intrinsic autofluorescence (produced by the physiological presence of autofluorescent molecules), using antibodies conjugated with horseradish peroxidase (HRP)-labelled secondary antibodies. We utilized multiple monoclonal and polyclonal antibodies, all from Dako (Carpinteria, California, USA). For all our IHC testing, we used a dual endogenous peroxidase blockage, with the addition of an Envision dual link (to assist in chromogen attachment). We applied the chromogen 3,3-diaminobenzidine, and counterstained with hematoxylin. The samples were run in a Dako Autostainer Universal Staining System. Positive and negative controls were consistently performed. Our studies were specifically performed as previously described [7-13].

Indirect immunoelectron microscopy (IEM):
Our technique was performed as previously described [7-13]. In brief, postembedding immunogold labeling was performed on samples of El Bagre-EPF sera and controls. Peripheral rat nerve was used as an antigen; the tissue was dissected, fixed in 4% glutaraldehyde with 0.2% paraformaldehyde, and embedded in Lowicryl® resin. The tissue was then sectioned at 70 nm thickness. The samples were blocked with a solution from Aurion™ (Electron Microcopy Sciences/EMS, Hatfield, Pennsylvania, USA). Our tissue grids were then washed with PBS-BSAC (Aurion™, EMS). The primary antibodies were incubated overnight at 4°C. The next day the grids were again washed, and a secondary antibody solution, specifically 10 nm gold-conjugated protein A PBS BSAC (Aurion, EMS™) was applied. The samples were then double-stained with uranyl acetate and lead citrate. The samples were observed under a Hitachi H7500 transmission electron microscope. Immunogold particles displaying any pattern of positivity were converted to TIF format.

Results

Histologic findings:
Examination of the H&E tissue sections demonstrated a focal, subepidermal blistering process in 6/7 of our biopsies. Specifically, multiple, punctuate subepidermal vesicles were present at the tips of the dermal papillae, with numerous neutrophils noted within the vesicular lumens in 7/7 cases. Occasional eosinophils were also present. In all biopsies, the papillary dermis contained a mild, superficial, perivascular infiltrate of lymphocytes, histiocytes and neutrophils; eosinophils were rare. No definitive evidence of an infectious, or a neoplastic process was observed. No dyskeratosis or acantholysis was noted. The control biopsy findings were negative for blisters and neutrophilic infiltrates.

Immunofluorescence studies:
DIF studies displayed the following positive results: IgG (+, in the dermatitis herpetiformis bodies and weakly positive granular staining at the BMZ) in 6/7 cases; IgG3 and IgG4 (+, diffuse positivity in the upper dermal papillae); IgA (+++, granular deposits at the BMZ in 7/7 cases, accentuated at the tips of dermal papillae; also in the DHBs); IgM, anti-kappa and anti-lambda light chains (++, positive in the dermatitis herpetiformis bodies, and granular deposits at the BMZ); IgD (-); IgE (++, several positive cells around dermal blood vessels); complement/C1q (+/-, granular deposits at the BMZ); complement/C3 (++, granular at the BMZ, and accentuated at tips of dermal papillae); albumin(+/-, granular at the BMZ) and fibrinogen (+++, granular at the BMZ, accentuated at tips of the dermal papillae and positive in the dermatitis herpetiformis bodies). The IIF utilizing monkey esophagus (ME) substrate (Oregon National Primate Center, Beaverton, Oregon, USA) showed positive endomysial antibodies, with both IgA and IgM in 6/7 cases. The dermatitis herperpetiformis bodies did not demonstrate colocalization with tissue/epidermal transglutaminase, which was positive in only 3/7 cases and in none of the controls

Immunohistochemical studies:
IHC demonstrated cells positive for Factor XIIIa in the papillary and reticular dermal interstitial tissue and around the upper dermal blood vessels in 3 biopsies. Several cells were also positive and displayed a similar dermal staining pattern with antibodies to myeloid/histoid antigen, mast cell tryptase (MCT) and c-kit (CD117). The CD1a marker was relatively normal, without evidence of a pathologic pattern (Fig. 1, 2). The figures display several representative photomicrographs showing von Willebrand factor (VWF) overexpression; antibodies to IgA, IgG, IgM, kappa and lambda light chains, fibrinogen, albumin, complement/C1q, complement/C3c, and complement/C3d were also positive in similar patterns. Of importance, the positive reactivity of these immunoglobulins and complement components was not only observed by IHC, but also confirmed via DIF and IIF.

Discussion

Given our data, we suggest that dermatitis herpetiformis bodies are complex structures composed of an agglomeration of several immunoglobulins (IgA, IgM and to a lesser extent, IgG). In addition, fibrinogen, complement/C3 and molecules that react with ARVCF [specifically, desmoplakins 1 and 2 and plakophilin 4 (p0071)] seem to be colocalizing with the dermatitis herpetiformis bodies. Previously, other authors have described deposits of fibrin within DHBs in some patients with DH [14,15].

It has been previously suggested that circulating IgA and IgG EmA and tTG antibodies are detected in almost all patients in the acute phase of dermatitis herpetiformis, and that these markers follow the clinical course of the disease. However, we were not able to colocalize these markers within DHBs. Other authors however have found an association between levels of IgA antibodies to tissue transglutaminase and gliadin-related nonapeptides in dermatitis herpetiformis [16].

We did find colocalization of desmoplakins 1 and 2, p0071 and ARVCF molecules with the dermatitis herpetiformis bodies. Several of these molecules are linked to neuro-vascular structures; perhaps these findings can explain the pruritus seen in DH patients. Of interest, several molecules of the armadillo repeat motif are necessary to maintain barrier function and intestinal homeostasis [17,18]. ARVCF, an armadillo repeat protein of the p120 catenin (ctn) family, associates with classical cadherins. Plakophilins 1-3 are also members of the p120 (ctn) family [17,18]. The plakophilins have been characterized as desmosomal proteins, while p120 (ctn) and the closely related delta-catenins ARVCF and p0071 associate with adherens junctions and play indispensable roles in stabilizing cadherin-mediated adhesion. Recent evidence suggests that plakophilins are essential components of the desmosomal plaque, where they interact with desmosomal cadherins as well as the cytoskeletal linker protein desmoplakin [17,18]. The three plakophilins exhibit distinct expression patterns, and although partially redundant in their function, mediate distinct effects on desmosomal adhesion. In our patients, we showed colocalization of several of these molecules [e.g. ARVCF, desmoplakins 1 and 2 and plakophilin 4 (p0071)] with the DH antibodies by both DIF and CFM. All of these molecules are present in the skin, heart, kidney, intestine and nerves.

Correlating our immunoblotting studies, our patients displayed autoantibodies with the same molecular weights (MWs) as the molecules we found to be colocalizing with the dermatitis herpetiformis bodies, including p0071 (135 kDa MW) ARCVF (97 kDa MW), and desmoplakins 1 and 2 (250 and 210 kDa MW, respectively). Therefore, we cannot exclude the possibility that patients with DH could have autoantibodies to these molecules. Similar reactivity has been recently described in patients with PNP that recognize plakophilin 3, a member of the p120 (ctn) family [19]. In addition, we showed that our patients displayed cytoid bodies. Cytoid bodies are predominately formed by IgM, and/or fibrin not colocalizing with the immunoglobulins, complement components and other molecules described above.

In addition, our and other authors' previously published articles suggest that our findings of autoantibodies to peripheral nerve axons may contribute to the clinical pruritus, metamerical grouping and flexoral distribution of DH lesions [20,21]. We can exclude that the neural reactivity was due to a side effect of dapsone [22], since this medication was initiated only after skin and blood samples were obtained from our patients. Recently, other authors also described the expression of selected neuropeptides in the pathogenesis of bullous pemphigoid and dermatitis herpetiformis [20]. Our findings using immunoelectron microscopy and immunogold antibodies clearly show deposits of autoantibodies in the peripheral nerves. We thus speculate that our findings may explain the metamerical, grouped and pruritic nature of DH lesions. Further studies are necessary to confirm our hypothesis.

In our patients, we also confirmed autoreactivity against the kidney; others have also described these associations [23,24]. Specifically, immunoreactivity to the kidney has been also previously documented in patients with DH exhibiting normal kidney function. Indeed, electron microscopy performed on some patients with DH who had no previous signs or symptoms of renal disease demonstrated mesangial deposits, and subsequent renal biopsy and DIF revealed IgA and complement deposits in the glomeruli. Renal involvement was not related either to the degree of jejunal villous atrophy, or to the deposition of IgA and complement in the skin. Glomerular deposits, however, were associated with a high frequency of circulating IgA and IgG subclass immune complexes and IgA subclass antigliadin and antireticulin antibodies [23,24].

Other authors have also found alterations within the heart associated with some patients suffering from DH [25,26]. We suggest that our findings may be pertinent in this context.

We also detected autoantibodies to heart and kidney mitochondria in the sera from patients affected by dermatitis herpetiformis. Other authors have described antimitochondrial autoantibodies in pemphigus vulgaris as a missing link in disease pathophysiology [27]. We also described antimitochondrial autoantibodies in a new variant of endemic pemphigus foliaceus in El Bagre, Colombia, South America [27].

Since DH is a rare disease, further studies with larger numbers of patients are needed to confirm our data regarding the nature of dermatitis herpetiformis bodies. Moreover, although transglutaminase has been described as a primary antigen for DH, it is possible that many other DH antigens remain to be discovered in the skin, other organs, and in mitochondria.

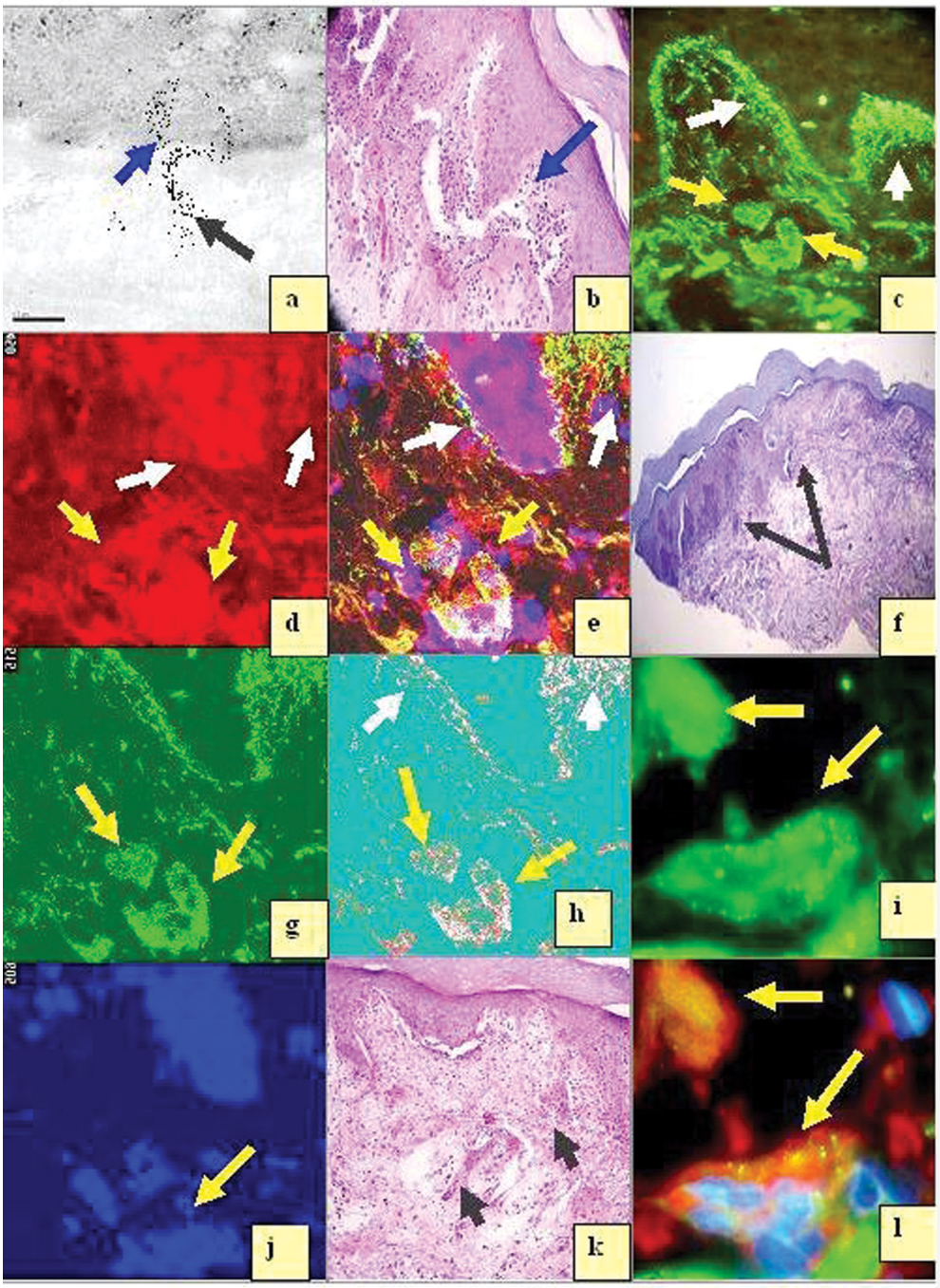

Figure 1. Dermatitis herpetiformis bodies detected using multiple test modalities

Figure 1. a IEM positive deposits of 10nm Gold particles near the BMZ of the skin; the black arrow highlights the staining at the BMZ, and the dark blue arrow shows staining in the upper papillary dermis. **b.** H&E staining demonstrating a DH subepidermal blistering process with dermal edema and multiple luminal neutrophils (blue arrow) (100x). **c.** DIF positive staining utilizing FITC conjugated anti-human IgA at the dermal papillary tip BMZ ("snow on the mountain" pattern; green staining, white arrows). Also note positivity on a DHB in the papillary dermis (green staining; yellow arrows). **d.** CFM utilizing Texas red conjugated anti-armadillo repeat deleted gene in velo-cardio-facial syndrome (ARVCF) antibody (Progen, Heidelberg, Germany) shows positive staining in the papillary dermis (white arrows) and in DHBs (yellow arrows). **e.** CFM confirming colocalization between FITC conjugated human IgA antibodies (green staining) and the Texas red conjugated ARVCF antibodies (red staining). The white arrows highlight colocalizations at the BMZ on the tips of the dermal papillae, and the yellow arrows highlight positivity in DHBs. **f.** PAS positive staining highlighted at the BMZ, where the primary inflammatory process is present (faint red staining; black arrows). **g.** CFM utilizing FITC conjugated anti-human IgA antibodies alone (green staining) showing positivity within dermal papillae on DHBs (yellow arrows). **h.** A CFM silhouette of e, showing colocalization of antibodies to IgA and ARVCF (white arrows at the BMZ, yellow arrows on DHBs). **i.** DIF showing staining of the DHBs using FITC conjugated anti-human IgA (green staining; yellow arrows) and in l, colocalizing this antibody with Texas red conjugated ARVCF (yellow arrows). In l, note the upper DHB contains no cell nuclear material, whereas the lower DHB contains blue nuclear material, counterstained with Dapi (4',6-diamidino-2-phenylindole). Note Dapi nuclear counterstaining of pertinent areas in **d, e, g,** and **h.** Please also note that some areas of the DHBs reveal no nuclear material, and other areas contain nuclear material. **j.** Same as g, with only Dapi staining. **k.** H&E shows eosinophilic material in the dermis where the DHBs are located (black arrows).

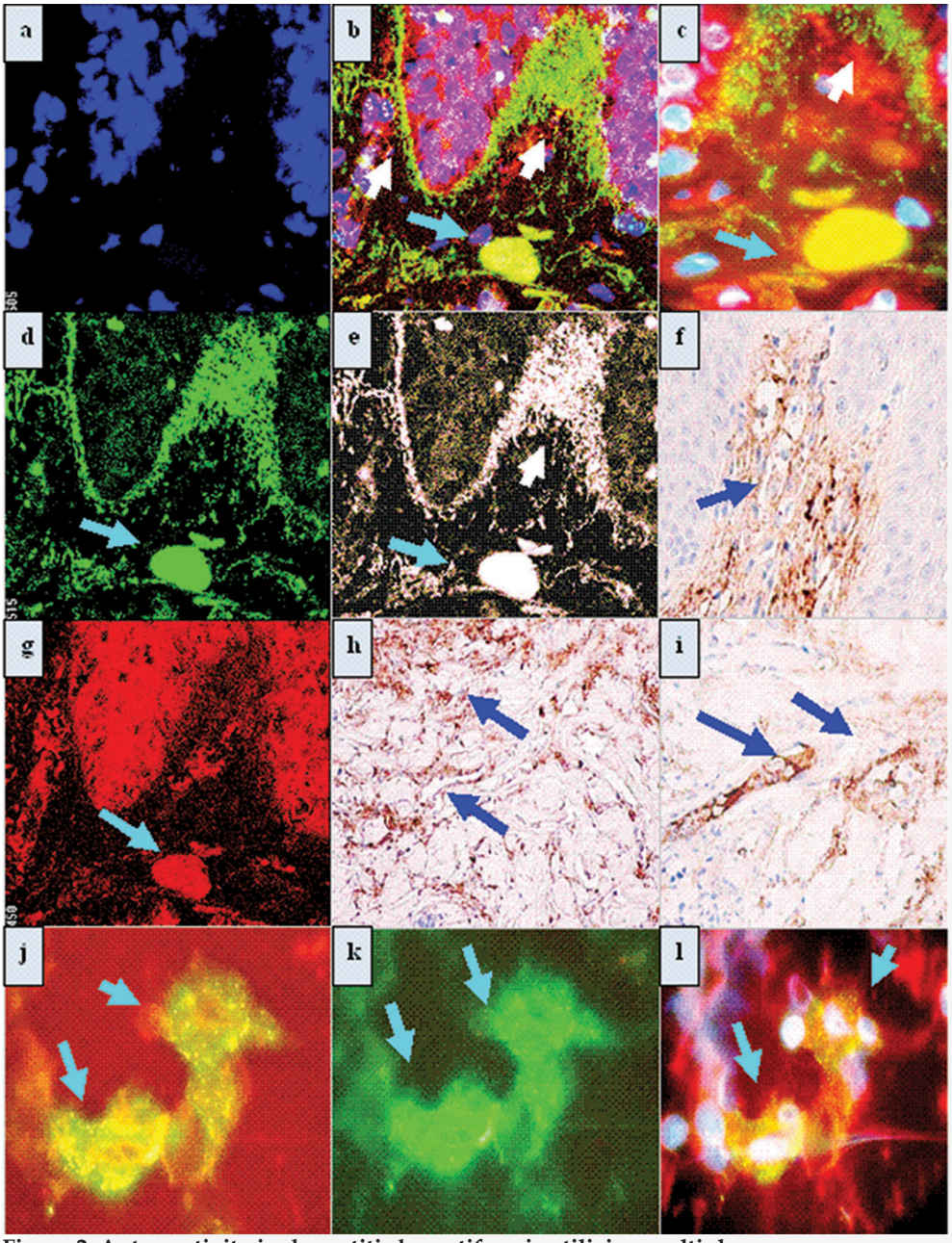

Figure 2. Autoreactivity in dermatitis herpetiformis utilizing multiple assays

Figure 2. Shows representative images demonstrating that both ARVCF antibody and an antibody against combined desmoplakins I and II (DPI-DPII) (Progen) stain the DHBs. Figures **a, b, d, e** and **g** represent CFM. **a.** Dapi counterstaining of epidermal keratinocyte nuclei in blue. **b.** Shows CFM colocalization of antibodies (FITC conjugated IgA in green, and Texas red conjugated DPI-DPII in red). The white arrows indicate positivity at the BMZ and dermal papillae; the light blue arrow to a DHB. **c.** DIF image of the IgA and DPI-DPII colocalization, showing similar results to **b. d** Positive CFM staining against the BMZ and dermal papillae, as well as to a DHB (light blue arrow) utilizing FITC conjugated anti-human IgG (green staining). **e.** A CFM silhouette of the overlapping antibodies in **b.** The white and light blue arrows are defined in **b. f** IHC staining with IgA on upper dermal blood vessels (brown staining, blue arrow). **g.** Positive CFM staining utilizing Texas red conjugated DPI-DPII (red staining); the light blue arrow points to a DHB. **h.** IHC staining of dermal blood vessels with anti-human IgM (brown staining; blue arrows); in **i,** similar IHC staining with anti-fibrinogen (brown staining; blue arrows). **j, k** and **l** demonstrate positive DHB DIF staining. In **j,** colocalized FITC conjugated anti human IgA (yellow-green staining) and Texas red conjugated anti-DPI-DPII antibodies (red staining) (light blue arrows). In **k,** DIF similar to the j image, utilizing only FITC conjugated anti-IgA (green staining; blue arrows); **l.** Similar to the **j** DIF image, but also including nuclear counterstaining with Dapi (white/blue staining, light blue arrows).

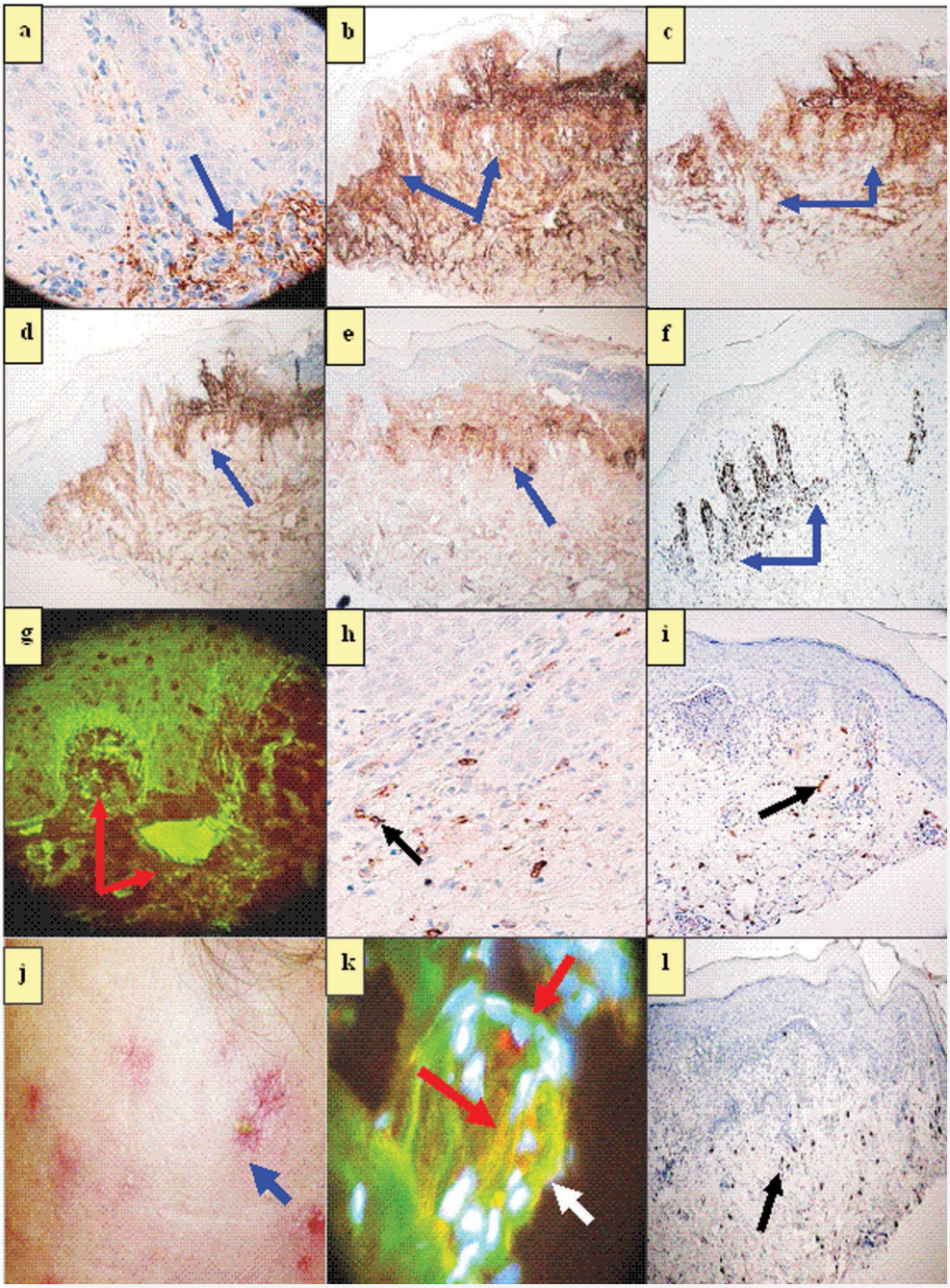

Figure 3. Clinical lesions, immunohistochemistry and immunofluoresence in dermatitis herpetiformis

Figure 3. a IHC positive staining for anti-human IgM around papillary dermal blood vessels (blue arrow, brown staining). **b.** Strong deposition of albumin detected in the dermis by IHC, in areas of the autoimmune reaction (brown staining, blue arrows). In **c,** strong IHC deposits of complement/C3c are noted in the same dermal areas as in **b** (brown staining, blue arrows); in **d,** robust IHC deposits of fibrinogen in these areas (following the pattern of positivity of IgM, C3c and albumin; brown staining, blue arrow). **e.** Shows that the highly reactive areas shown in **a** through **d** also have simultaneous IHC overexpression of von Willebrand factor (VWF) (brown staining, blue arrow). Please note that there is differential expression (or compartmentalization) of the VWF positivity; the expression is seen primarily in the papillary dermis, with a more delicate presence in the subjacent reticular dermis. **f.** IHC staining with compartmentalization of the autoimmune response, utilizing myeloid/histoid antigen antibody; concentrated staining is noted in the dermal papillae (black staining, blue arrows) **g.** DIF also shows positive staining for FITC conjugated anti-IgM (green staining) at the BMZ, as well as in the papillary dermis and on a DHB (red arrows). **h.** Positive IHC staining of multiple individual dermal cells with anti-IgE, especially around dermal blood vessels (brown staining, black arrow). **i.** We also detected several positively stained cells with anti-Factor XIIIa in the dermis by IHC (brown staining, black arrow). **j.** A classic clinical lesion, showing ruptured, punctate blisters on an erythematous, macular base (blue arrow). **k**. Colocalizing positive DIF staining against a dermal nerve subjacent to a clinical blister and inflammation. The colocalization is performed utilizing FITC conjugated anti-human-IgM (green staining, white arrow) and Cy3 conjugated monoclonal anti-glial fibrillary acidic protein (GFAP) (red-orange staining, red arrows). **l.** IHC staining with mast cell tryptase (MCT) around dermal blood vessels at the periphery of the primary autoimmune response (brown staining, black arrow).

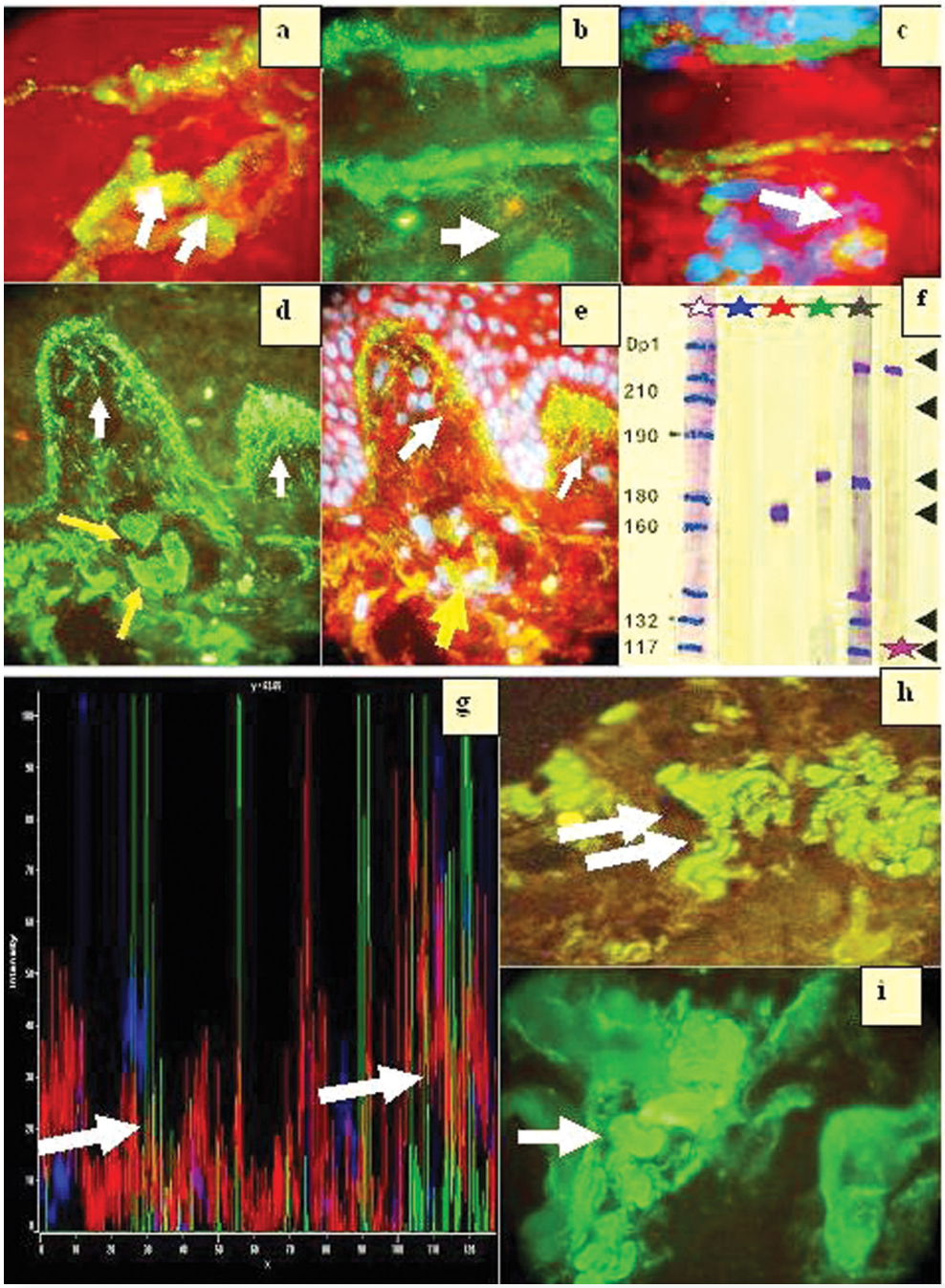

Figure 4. Immunologic findings, immunoblotting and confocal microscopy in dermatitis herpetiformis

Figure 4. Shows some representative pictures with colocalization of patient autoantibodies and p0071 antibodies, differentiating the DHBs from cytoid bodies. In **a,** DIF positive staining with FITC conjugated IgA at the BMZ and against a DHB (yellow-green staining) colocalizing with Texas red conjugated p0071 antibody (red staining) (white arrows). Note the staining within the DHB appears as multiple dots. **b,** Similar to **a,** but only with anti-IgA, without staining for p0071 (yellow staining; white arrow). **c.** Similar to **a,** but with additional nuclear counterstaining with Dapi (blue staining), demonstrating that the dermatitis herpetiformis bodies contain nuclear debris (white arrow). **d** and **e,** lower magnification images of **b** and **c,** respectively, showing that the dermatitis herpetiformis bodies (yellow arrows) represent discretely different staining structures vis-a-vis nearby staining to the papillary tips and BMZ (white arrows). **f.** Immunoblotting; the first lane (white star) shows the reactivity of a paraneoplastic pemphigus serum used as a positive control. Desmoplakin is represented by the highest band. The second lane (blue star) is a negative control. The third lane (red star) is a serum positive control, from a patient affected with pemphigus foliaceus (note positivity for the 160 kDa antigen, desmoglein 1). The fourth column (green star) is a positive control serum from a patient affected with bullous pemphigoid (note the bullous pemphigoid 2 antigen at 180 kDa). The fifth column (brown star) is one of the patient's DH serum; please note the presence of bands around 250-230 kDa; these bands represent desmoplakins, confirmed by the sixth column. In the fifth column, please also notice that three additional bands of 140, 132 and 117 kDa are present. The sixth column (bottom pink star) is a control monoclonal desmoplakin protein (Progen). **g.** CFM data, demonstrating precise colocalization of several peaks of the Texas red conjugated antibody to p0071 (red peaks) with FITC conjugated IgA (green peaks) (white arrows). The blue peaks represent Dapi nuclear counterstaining. **h** and **i** Globular cytoid body deposits of immunoglobulins; in **h,** FITC conjugated IgM (yellow staining, white arrows), and in **i,** FITC conjugated fibrinogen (green staining, white arrow). These cytoid body findings did not colocalize with other antibodies staining the DHBs (i.e., p0071, ARVCF, and IgA).

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