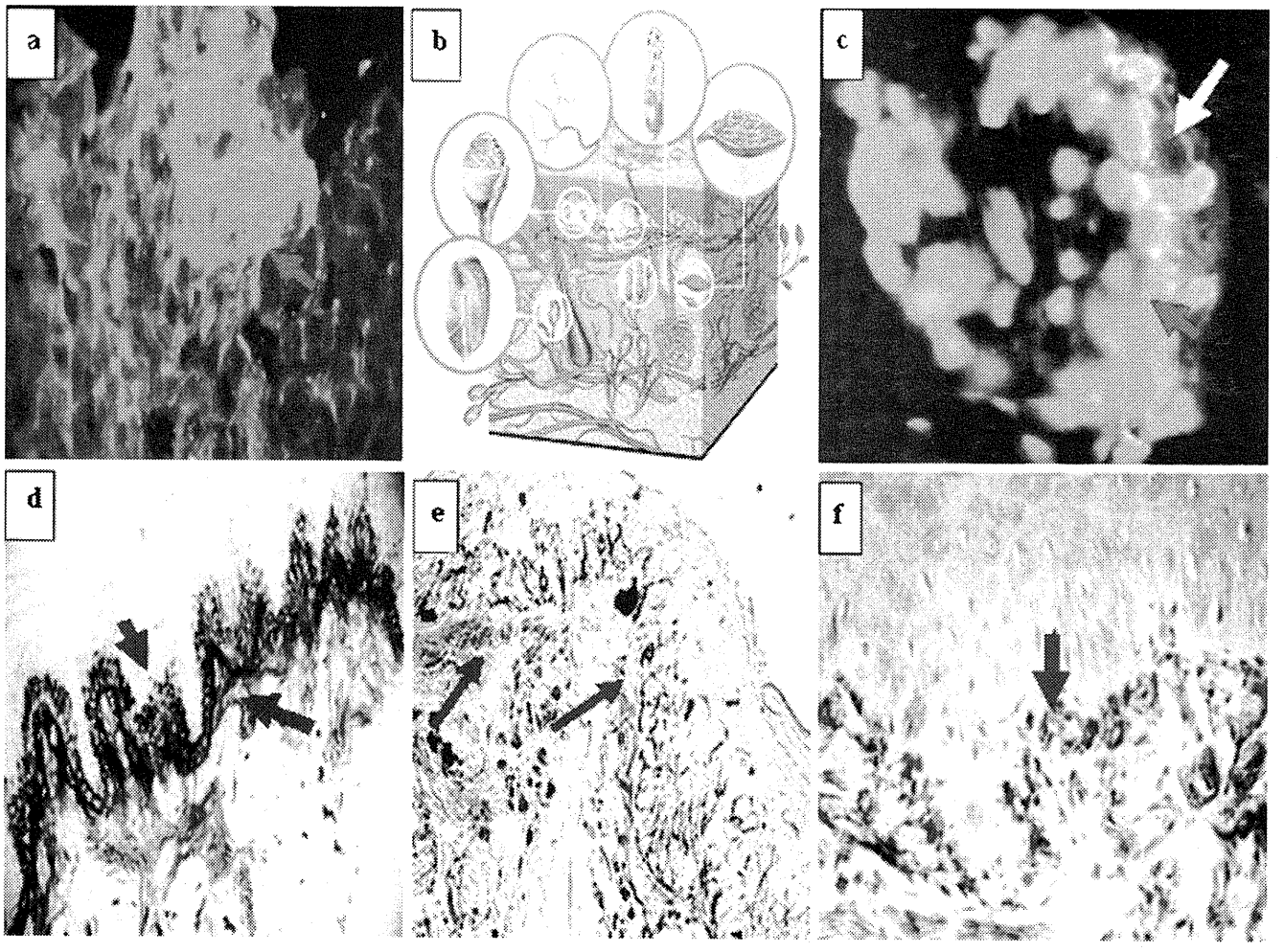


Fig. 3 El Bagre-EPF patient sera recognize other mechanoreceptors, and their skin biopsies show nerve paucicellularity and defragmentation of small nerves. "Pose of pemphigus"

human IgG/IgM/IgA antiserum. In contrast, two of the 20 normal controls from the endemic area showed autoreactivity to larger nerves with only IgM.

In Fig. 3, El Bagre-EPF patient sera recognize other mechanoreceptors, and their skin biopsies show nerve paucicellularity and defragmentation. Figure 3a shows positive staining of a Krause endbulb using FITC-conjugated anti-human IgM antibodies (red arrow) (green staining). Figure 3b is a diagram of mechanoreceptors of the skin. Figure 3c shows positivity with FITC-conjugated anti-human fibrinogen antiserum (red arrow, green staining), and with anti-human-ICAM-1/CD54 (orange staining, white arrow) in a Meissner corpuscle. The nuclei are counterstained with Dapi (blue). Figure 3d shows normal silver staining of the upper neural plexus of the skin of a normal control (dark brown netting stain; blue arrows). Figure 3e shows silver staining from one El Bagre-EPF skin biopsy, revealing defragmentation and loss of nerves (blue arrows). Figure 3f shows IHC of one El Bagre-EPF skin biopsy, utilizing anti-human C1q antibodies positive against the upper neurovascular bundle under the BMZ with anti-human C1q (blue arrow).

In Fig. 4. El Bagre-EPF patient sera recognize thin myelinated and non-myelinated nerves, the spindle cell apparatus, and brain tissue by different techniques. Figure 4a shows perineural inflammation and mild edema of a nerve in an H&E skin biopsy of an El Bagre-EPF patient (×100). Figure 4b and d is DIF showing positive colocalizations of El Bagre-EPF patient's autoantibodies using FITC conjugated-anti-human IgG antibodies (in Fig. 4b, to mechanoreceptors; in Fig. 4d, to the neuromuscular spindle; we used anti-GFAP to demonstrate the neural nature of these structures). The Bagre-EPF antibodies are shown in green staining (red arrows). In Fig. 4d, the red-orange stain shows El Bagre-EPF atutoreactivity to the neural spindle (yellow arrows). Figure 4c shows IB using cow spinal cord extract. On the left are the molecular weight standards and on the right is a positive control. After SDS polyacrylamide gel electrophoresis separation of cow spinal cord and blotting of neural substrate antigens, El

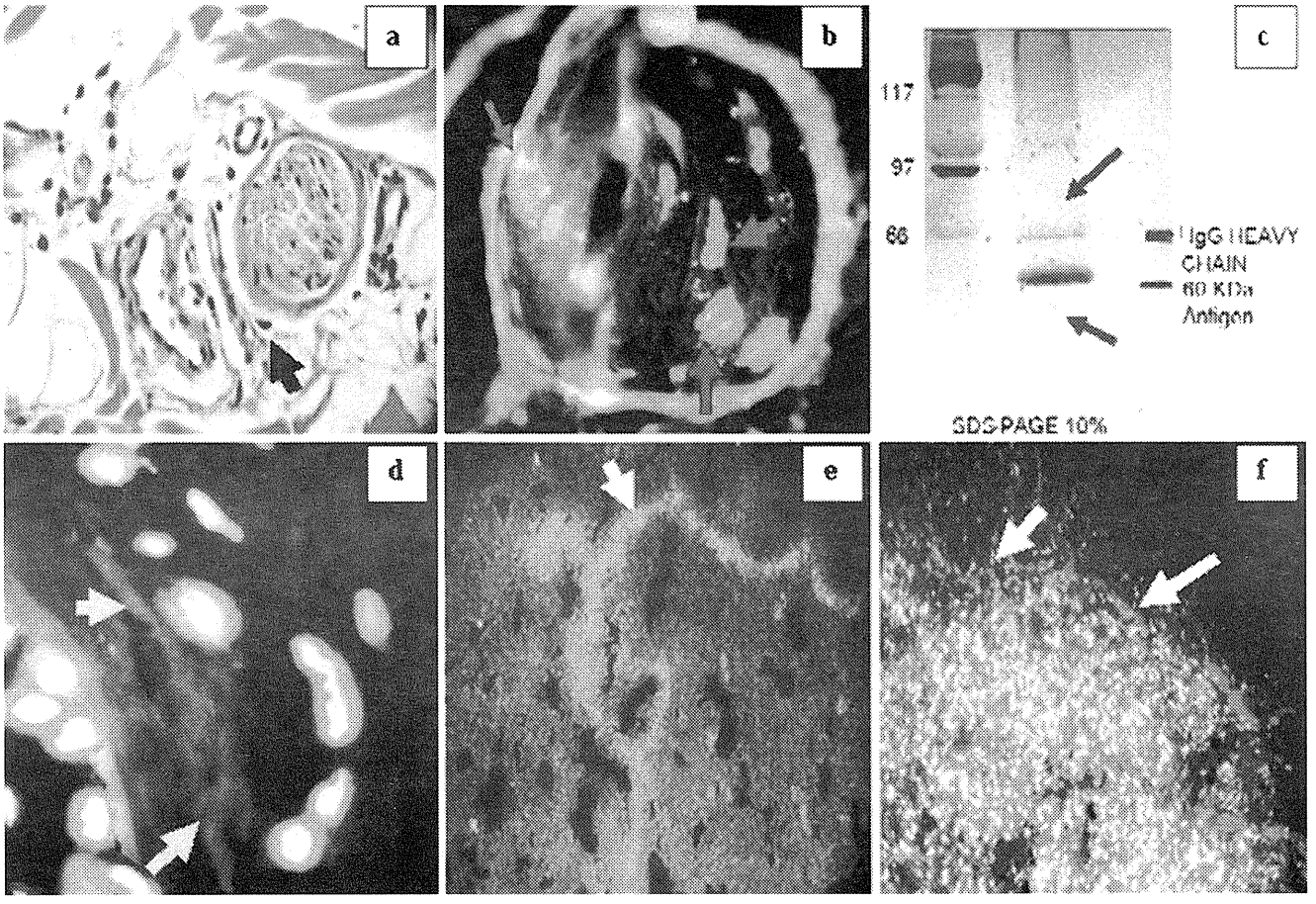


Fig. 4 El Bagre-EPF patient sera recognize myelinated and non-myelinated nerves, the spindle cell apparatus, and some brain tissues by different techniques

Bagre-EPF sera reactivity to several bands are shown, including 135, 97, 66 and 60 kDa(the last two, highlighted with blue arrows, were correlated using anti-human-IgG and IgM, respectively). We found that one third of the El Bagre-EPF sera were reactive to some of these four bands. No controls from outside or inside the endemic area were positive. Figure 4e illustrates positive FITC-conjugated IgG from an El Bagre-EPF patient, showing positive reactivity to the rat brain gyral and the sulci surface (green staining; white arrow). In Fig. 4f, similar pattern is seen with DPI-II (red staining; white arrows). We were able to demonstrate that El-Bagre-EPF patient autoantibodies colocalized in several areas of the brain and neurovascular structures with the commercial antibodies from Progen directed to DPI and DPII, to ARVCF, and with the P0071 antibodies (data no shown).

In Fig. 5, El Bagre-EPF patient autoantibodies colocalize with neural markers using IEM, CFM, and IHC. Figure 5a, c demonstrates colocalization of the neural components with El Bagre-EPF patient sera using FITC-conjugated human IgM and IgA antibodies (white staining; yellow arrows) and Texas red-conjugated GFAP (orange staining) (red arrows). In Fig. 5b, El Bagre-EPF autoantibodies colocalize using FITCI-conjugated human-IgM antibodies (yellow staining; red arrows). In Fig. 5d, e, CFM demonstrated that the El Bagre-EPF antibodies colocalized with neural markers. Figure 5d shows CFM image revealing anti-GFAP (red), anti-IgG (green), and Dapi (nuclei; blue) in their respective staining patterns, utilizing the EZ 1 Viewer software for image analysis. The light gray represents the overlapping of GFAP and IgG using NDIC. In Fig. 5e, we measured the staining of the overlap distances and found colocalization of the dyes in the same focal plane. The GFAP and the IgG peaks overlap (white arrows), in contrast to the Dapi (yellow arrow). Figure 5f, g shows IEM images. We observed 10 nm gold particles, representative of El Bagre-EPF autoantibodies deposited in the axons of the peripheral nerves at lower magnification (Fig. 5f, 60 kV) and at higher magnification (Fig. 5g, 150 kV) (red arrows, black particles).

Table I shows mercury levels in the patients and controls and documents neural alterations found in comparison with mercury levels at the time of the examination.

Discussion

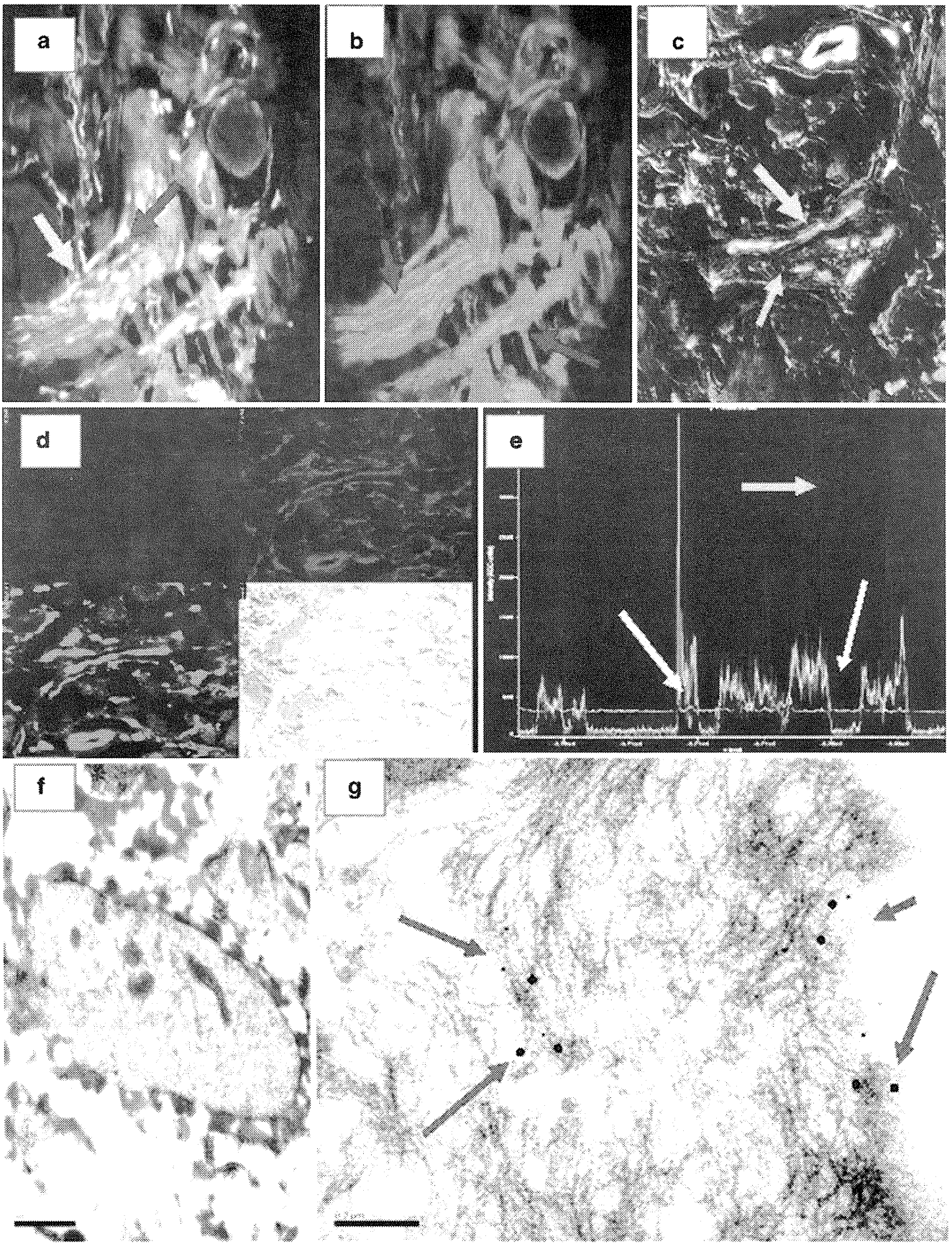
Patients affected by FS and El Bagre-EPF have burning sensation on the skin, combined with itching and occasional paresthesias statistically significant even comparing with the controls living in the same endemic area where mercury and other metals and metalloids prevail [4, 5, 7, 18]. Other neuromuscular symptoms reported before the steroid era for patients affected chronically by EPF included depression, mood disturbances, decalcifications, muscular atrophy (most of extensors muscle), contractual deformities, and ankylosis of the joints producing the "pose of pemphigus" (dosiflexion of extremities) [1–8]. Based on our studies, we can speculate that the "pose of pemphigus" is explained vis-a-vis for the weakness and/or direct damage of the extensor nerves for an unknown reason to us yet [1–12]. Our findings of polyclonal neural reactivity by colocalization of El Bagre-EPF autoantibodies with neurovascular markers could explain the skin burning and "be in fire sensation" symptoms and could provide valuable clues to the pathophysiological process. In addition, antibodies to the spindle cells of the neuromuscular apparatus may explain the clinical neuromuscular atrophy and the increased dorsiflexural tone in the "pose of pemphigus". Furthermore, by IB, we detected reactivity of several molecules, including plakins and unknown molecules of 135, 97, 66, and 60 kDa in several sera from El Bagre-EPF patients. Of interest, p0071 is 135 kDa in molecular weight, and ARVCF approximates 97 kDa. IB revealed that IgM and IgG antibodies correlated with our DIF, IIF, and IHC testing.

We detected (1) autoantibodies to mechanoreceptors and mostly to thin nerves of the skin including some myelinated and some not. We also detected autoantibodies to the optic nerve, but mostly to the perineural meningeal sheaths [that are rich in desmoplakins (El Bagre-EPF antigens)]. In addition, we detected neural paucicellularity, decreases in the ENFD, and autoreactivity to parts of the brain that all colocalized with the patient's autoantibodies and several neural markers. We demonstrated colocalization of the patient's autoantibodies with DPI and DPII (known to be antigens for El Bagre-EPF) in the optic nerve and brain tissues. We recently described several ocular abnormalities and the presence of autoantibodies to the meibomian and other structures of the eyes in El Bagre-EPF patients [6]. Desmosomes are major intercellular junctions found in association with intermediate filaments in epithelial, cardiac, and arachnoidal tissue that surrounds the optic nerve [19]. DPI and DPII are part of the desmosomal plaque and seem to have a role for linking intermediate filaments in several tissues [19]. DPI and DPII are not restricted to stratified epithelia [19]. Interestingly, ARVCF is associated with E-, M-, and possibly N-cadherins [20]. Thus, we suggest that epitope spreading could occur in the disease process in El Bagre-EPF patients.

Within the skin, we observed defragmentation and alterations in the neural plexuses by several methods. Skin biopsy has been demonstrated to be of value in the diagnosis of clinical small fiber neuropathies; this technique is less invasive than nerve biopsy [12–15]. In autonomic neuropathies, diagnostic fibers are located in the dermis; thus, fibers innervating sweat glands and piloerector muscles can be assessed [12–15]. We recently described autoantibodies to sweat glands in patients affected by El Bagre-EPF [21]. Here, we demonstrate antibodies to multiple neurovascular areas. Thus, we have demonstrated physical and immunological evidence of autoreactivity to the peripheral and central nervous system using multiple techniques. However, the neural symptoms could result from a combination of sympathetic and parasympathetic nerves, both myelinated and non-myelinated damage, and therefore, larger and extended studies need to be pursue to answer these questions.

We speculate that damage to the nerves may occur because intraepidermal nerves exist in proximity to desmosomes; blisters, acantholysis, and separation would occur in these areas, potentially exposing neural antigens to autoreactivity. A similar process could occur at the neurovascular plexus below the basement membrane zone or within the dermal papillae. Additionally, mercury, other elements, and/or other diseases could induce epitope mimicry and/or alter the conformation of selected molecules, thus triggering the autoimmunity. p0071 is very homologous to the neural plakophilin-related armadillo repeat protein (NPRAP/δ catenin) and is located in several cell junctions [22]. p0071, DPI, and DPII are part of the complexus adherens meshwork, related to endothelial and lymphatic cells [23]. These cells are connected by the complexus adherens, with VE-cadherin joining with DPI and DPII, as well as several adherens junction plaque proteins, such α- and β-catenin, p120 catenin, and components of tight junctions, such claudin-5, JAM-A, and ZO-1 [23].

Most current literature and trends have clearly showed that most autoimmune skin blistering disease, such pemphigus and pemphigoids, are for the known desmogleins and BP18 and BP230. However, alterations of the nerves or other neural structures have been previously described in bullous diseases and loss over time, including a patient with paraneoplastic pemphigus (PNP) with a pseudotumor of the spinal nerve [24, 25]. Another case of PNP was associated with a myofibroblastic tumor [26]. Two Russian studies have shown alterations in adrenergic and cholinergic nerves of the skin in patients suffering chronic pemphigus [27, 28]. Changes in the Gasserian ganglion have been identified in cases of oral pemphigus [29]. Kaposi reported that many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◀ **Fig. 5** Immunoelectron microscopy reveals deposits of El Bagre-EPF patient autoantibodies within nerve axons, and CFM and IHC demonstrate colocalization with neural markers

cases of fatal pemphigus showed anatomic changes of chronic myelitis in the spinal cord and/or in the sympathetic nerves [30]. Li et. al. reported that sera from a patient with BP with neurological changes recognized BP antigens by IB in the skin and brain [31]. In mice lacking the BPAG1 gene, neurons exhibited perturbations in their intermediate filaments and microtubules, leading to swellings and changes in the axons [31]. Our studies demonstrated autoantibodies by IEM using 10-nm gold particles in the axons of peripheral nerves; further IEM studies will be performed on the central neural system. Additional evidence of the importance of the neural system in pemphigus has been demonstrated by the detection of human alpha-acetylcholine and cholinergic receptors as pemphigus vulgaris antigens [32, 33].

Chronic exposure to mercury produces neural tissue alterations, as seen in acrodynia Minamata disease and in animal models exposed to mercury [34–36]. Symptoms typically include sensory impairment (vision, hearing, and speech), ataxia, disturbed sensation, and a lack of coordination. The type and degree of symptoms exhibited depend upon the individual toxin, the dose, and the method and duration of exposure [34–36]. These alterations differ from the severe burning sensation seen in El Bagre-EPF patients. Organic mercury affects primarily sensory peripheral nerves, with swelling and degeneration of Schwann cells and changes in both myelin sheaths and axons [32–35] Also differing, the skin biopsies from El Bagre-EPF showed several inflammatory cells as reported before and showed here [5, 8]. Most of autoantibodies produced by chronic mercury exposure alone in human and animals have been reported to be antinucleolar, differing to our findings.

In El Bagre-EPF patients, we have documented autoantibodies in the axons by IEM. Since autoantibodies to neural structures following exposure to mercury, metalloids or organophosphates are primarily directed against MBP and GFAP and of IgG, IgA, and IgM subclasses, a mercury association cannot be excluded in El Bagre-EPF etiology [34–36]. However, mercury exposure alone is not sufficient to develop pemphigus. In Minamata disease, no patients have been described with EPF [34–36]. In addition, in the control group from the endemic area, the predominance of any autoreactivity was seen using IgM and present only in some individuals; in contrast, most of the neurological alterations and the skin burning sensations presented exclusively in El Bagre-EPF patients. Finally, the autoantibodies in the patients were expressed against perineurium,

Table I Mercury levels in the patients and controls and neural alterations found in comparison with mercury levels at the time of the examination

Subjects	Disease Course	Range of levels of mercury detected in nails and hair in parts per million (ppm) where (+ mild, ++ moderate and +++ severe)	Presence of autoantibodies to neural structures, and isotypes of the antibodies	Skin burning sensation	Kinesiological and neurological alterations
El Bagre-EPF cases	Acute (<6 months after disease onset)	40% (+++) 20% (++) 20% (+) 20% (5+) 0% (0)	None	Positive in 100% cases	Fine tremor 50%
El Bagre-EPF cases	Chronic cases(>3 years after disease onset)	40% (+++) 20% (++) 20% (+) 20% (5+) 0% (0)	IgM alone (40%) IgA alone (40%) IgA. IgM, fibrinogen, C3 and IgG (70%)	Positive in 100% cases	Depression (100%)
Controls living in the endemic area	<6 months	30% (+++) 20% (++) 10% (+) 20% (5+) 20% (0)	None	Negative in 100% cases	Fine tremor 50%
Controls living in the endemic area	>3 years	40% (+++) 20% (++) 20% (+) 20% (5+) 0% (0)	IgM 1 control	Negative in 100% cases	Depression (30%)
Controls from out of the endemic area		100% (0)	None	Negative in 100% cases	None

epineurum, endoneurium, and blood vessels Both the NHP and the LKS clinical evaluation scale results correlate with our findings [16, 17]. We note that most of our affected nerves innervated extensor muscles; the significance of this finding is beyond the extent of our current study.

A paraformaldehyde prefixation of skin biopsies improved our assessment of neural tissues; we suggest larger studies to assess technique efficacy. Furthermore, the El Bagre-EPF patient focus exists in a rural area, with minimal medical resources. Ideal testing would address specific antigens with neural microarrays, quantitative sensory nerve testing (for large and small fibers), quantitative sudomotor axonal reflex testing, single photon and positron emission tomography, and electromyography.

Plakins are the major El Bagre-EPF antigens [1–12]. Homozygous mice with a desmoplakin gene ablation and subsequent rescue utilizing extra-embyonic ectoderm have shown that desmoplakin is important in embryonic development, affecting heart, neuroepithelium, skin, and vasculature integrity [37, 38]. Finally Balo and Foldavari [38] reported in 1948 that in the Gasserian ganglia and in other spinal ganglia, a chronic inflammatory process with perivascular infiltration, vacuolar degeneration of ganglionic cells, and proliferation of amphicytes [38] occurs. In addition, there is the presence of some granulomas, which were found at the juncture of posterior roots and spinal ganglia. These and other authors speculated that the disturbance of fluid circulation is caused by the granulomas consisting of arachnoidal cells. Our findings of autoreactivity against the arachnoid envelope antibodies may explain these findings. In summary, we suggest that neural autoreactivity may contribute to burning skin sensations, paresthesias, and the "pose of pemphigus" encountered in patients with EPF.

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OBSERVATION

Paraneoplastic Pemphigus Herpetiformis With IgG Antibodies to Desmoglein 3 and Without Mucosal Lesions

Renata Prado, MD; Sylvia L. Brice, MD; Shunpei Fukuda, MD; Takashi Hashimoto, MD; Mayumi Fujita, MD, PhD

Background: Pemphigus herpetiformis (PH) is a rare clinical entity that combines the clinical features of dermatitis herpetiformis and the immunopathologic features of pemphigus. The target antigen is usually desmoglein 1, with exceptional cases manifesting autoantibodies against desmoglein 3. More recently, it has been found that many patients with PH also demonstrate autoantibodies against desmocollin. The association of PH with a malignant neoplasm is rare.

Observations: We describe a patient with PH and a lung neoplasm. Immunologic studies demonstrated IgG antibodies to desmoglein 3 and to an unknown 178-kDa protein but no antibodies to desmocollin.

Conclusions: The association of PH with a thoracic malignant neoplasm has been reported in only 4 previous cases, and the neoplasm could be responsible for the unusual immunologic profile in the patient described herein. To our knowledge, this is the first report of PH with an associated neoplasm in which only anti–desmoglein 3 antibody was detected.

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PEMPHIGUS HERPETIFORMIS (PH) is a rare clinical entity that combines the clinical features of dermatitis herpetiformis and the immunopathologic features of pemphigus. It affects patients aged 30 to 80 years and manifests as a subacute onset of erythematous urticarial plaques and clusters of vesicles in a herpetiform arrangement. The mucous membranes are usually not involved. Histologic findings of PH include eosinophilic spongiosis with various degrees of acantholysis, which is usually minimal.[1-3] Eosinophilia may be present in some patients.[4,5] Direct immunofluorescence (IF) shows IgG antibodies on keratinocyte cell surfaces, and the target antigen has been shown to be desmoglein (Dsg) 1 in most cases,[3,6] with a few patients demonstrating autoantibodies to Dsg3.[1,3,6-8] Anti-Dsg antibodies in PH are thought to induce spongiosis with eosinophil infiltration but rarely produce acantholysis, in contrast to classic pemphigus. Although the association of cancer with immunobullous diseases can be seen in patients with paraneoplastic pemphigus (PNP) and pemphigus vulgaris (PV),[9-11] it is rare in patients with PH. We describe a patient having PH with IgG autoantibodies to Dsg3 and to an unknown 178-kDa protein associated with pulmonary neoplasia.

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REPORT OF A CASE

A 68-year-old Hispanic woman was seen with a 2-week history of diffuse pruritic lesions on the trunk and extremities. She denied any mucosal involvement or history of similar lesions. Her medical history was positive for hypothyroidism secondary to treated Graves disease, benign mucinous cystadenoma of the ovary after total hysterectomy, osteoporosis, and smoking. Her medications included levothyroxine sodium, calcium carbonate, vitamin D, alendronate sodium, aspirin, albuterol sulfate nebulization, and fluticasone propionate and salmeterol xinafoate inhaler. A review of systems was positive for decreased appetite, weight loss (12 kg in 2 years), shortness of breath on exertion, and constipation. On physical examination, several large erythematous annular plaques with central crust and scales were noted on the trunk and extremities. The head and neck were spared. The lesions ranged from 1 to 10 cm, and some lesions were coalescing (**Figure 1** A and B). Intact blisters with serous content were present on the proximal right anterior thigh and left calf. There were no mu-

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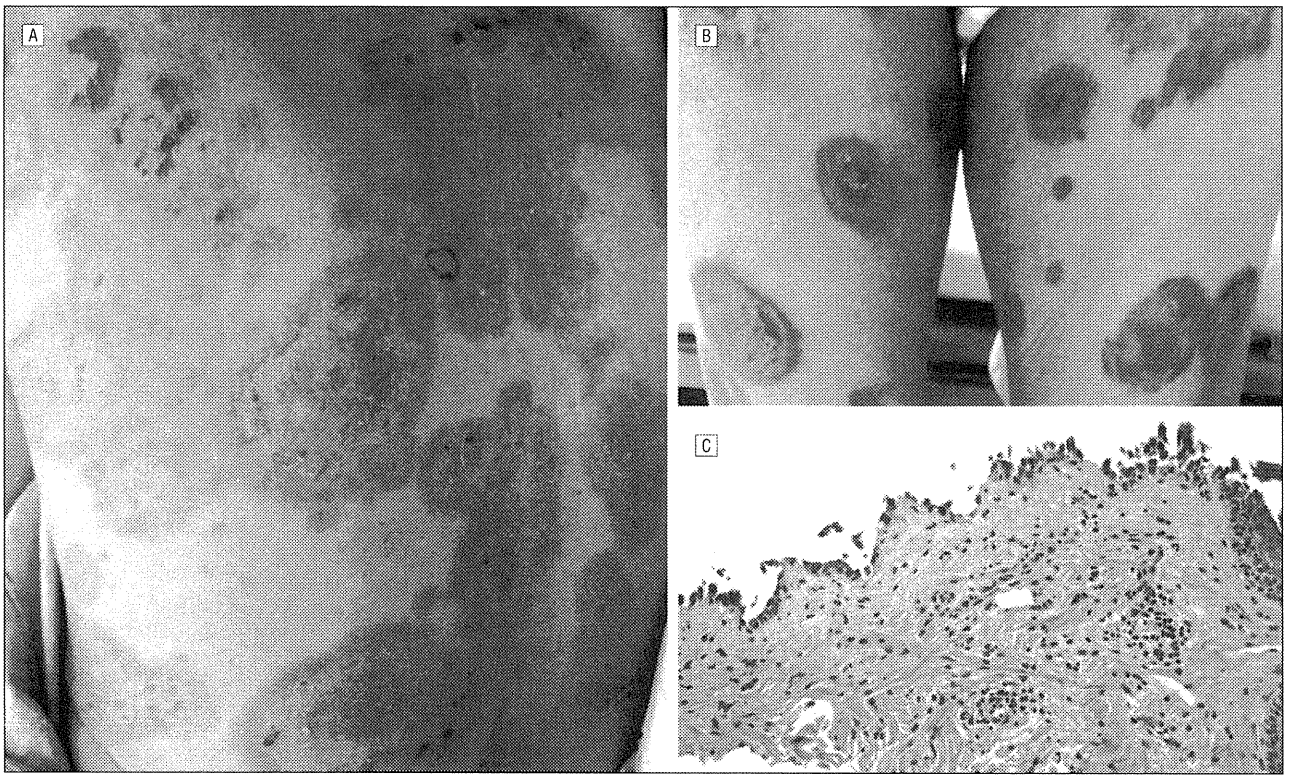

Figure 1. Large erythematous annular plaques with central crust and scales on the trunk and extremities. A and B, Some plaques contain vesicles in a herpetiform arrangement. C, Hematoxylin-eosin staining demonstrates an intraepidermal suprabasilar vesicle with a single row of keratinocytes attached to the basement membrane and a mild inflammatory infiltrate with eosinophils in the superficial dermis (original magnification ×10).

cosal lesions. Hematoxylin-eosin staining of skin demonstrated an intraepidermal suprabasilar vesicle with a single row of keratinocytes attached to the basement membrane and acantholytic keratinocytes within the vesicle. The superficial dermis contained a mild inflammatory infiltrate with eosinophils (Figure 1C). Direct IF demonstrated IgG deposition on the cell surfaces of keratinocytes. Indirect IF detected IgG antibodies at a titer of 1:100 that bound to the cell surfaces of monkey esophagus epithelium.

Enzyme-linked immunosorbent assay (ELISA) demonstrated marked elevation of autoantibodies to Dsg3 (193 U/mL). ELISA was negative for IgG antibodies to Dsg1, bullous pemphigoid (BP) 180, and BP230. The clinical presentation and histologic and laboratory findings were consistent with PH. The patient's condition improved significantly with systemic corticosteroid therapy, with almost complete clearing of the lesions within 1 week.

Laboratory studies revealed mild normocytic anemia, with a hemoglobin level of 11.1 g/dL (to convert hemoglobin level to grams per liter, multiply by 10.0), no eosinophilia, and normal results on thyrotropin, basic metabolic panel, and hepatic function tests. Her electrocardiogram was normal. Further evaluation of her weight loss, shortness of breath, and decreased appetite was recommended, but the patient did not undergo a chest radiograph until 4 months later, when it revealed a 7×6.5-cm mass on the mid right lung without pleural effusion. Chest computed tomography with intravenous contrast was then performed and demonstrated a large mass in the lower right upper lobe and right middle lobe with central necrosis. Right hilar masses consistent with lymph nodes were also found. The pulmonary artery branches were found to be obliterated around this mass, and probable tumor thrombus seemed to extend into the left atrium likely through the right superior pulmonary vein, forming an intra-atrial polypoid structure. Signs of severe emphysema were also noted. An attempt was made to contact the patient with the results the next day, but the patient had died.

To further characterize the immunologic profile of this patient, ELISA and IF using transfected COS-7 cells for desmocollin (Dsc),[12,13] as well as immunoblotting using normal human epidermal extracts as the source of antigens,[14] were performed. ELISA using antibodies against recombinant proteins of the entire extracellular domains of human Dsc1, Dsc2, and Dsc3 expressed by a baculovirus system was negative for IgG anti-Dsc1, anti-Dsc2, and anti-Dsc3 antibodies (the optical density cutoff value was 0.15). Immunofluorescence of transfected COS-7 cells also confirmed the absence of antibodies against Dsc1, Dsc2, and Dsc3 in our patient's serum: whereas a control serum sample from a patient with atypical pemphigus, who was known to have IgG anti-Dsc1, anti-Dsc2, and anti-Dsc3 antibodies, demonstrated granular staining of the cell surface; no staining was observed in COS-7 cells incubated with our patient's serum (**Figure 2**). Immunoblotting demonstrated IgG autoantibodies against a 130-kDa antigen (which corresponded to Dsg3 when using a serum sample from a patient with PV as a positive control) and against an unknown 178-kDa protein (**Figure 3**). The patient's serum was negative for autoantibodies against the BP230,

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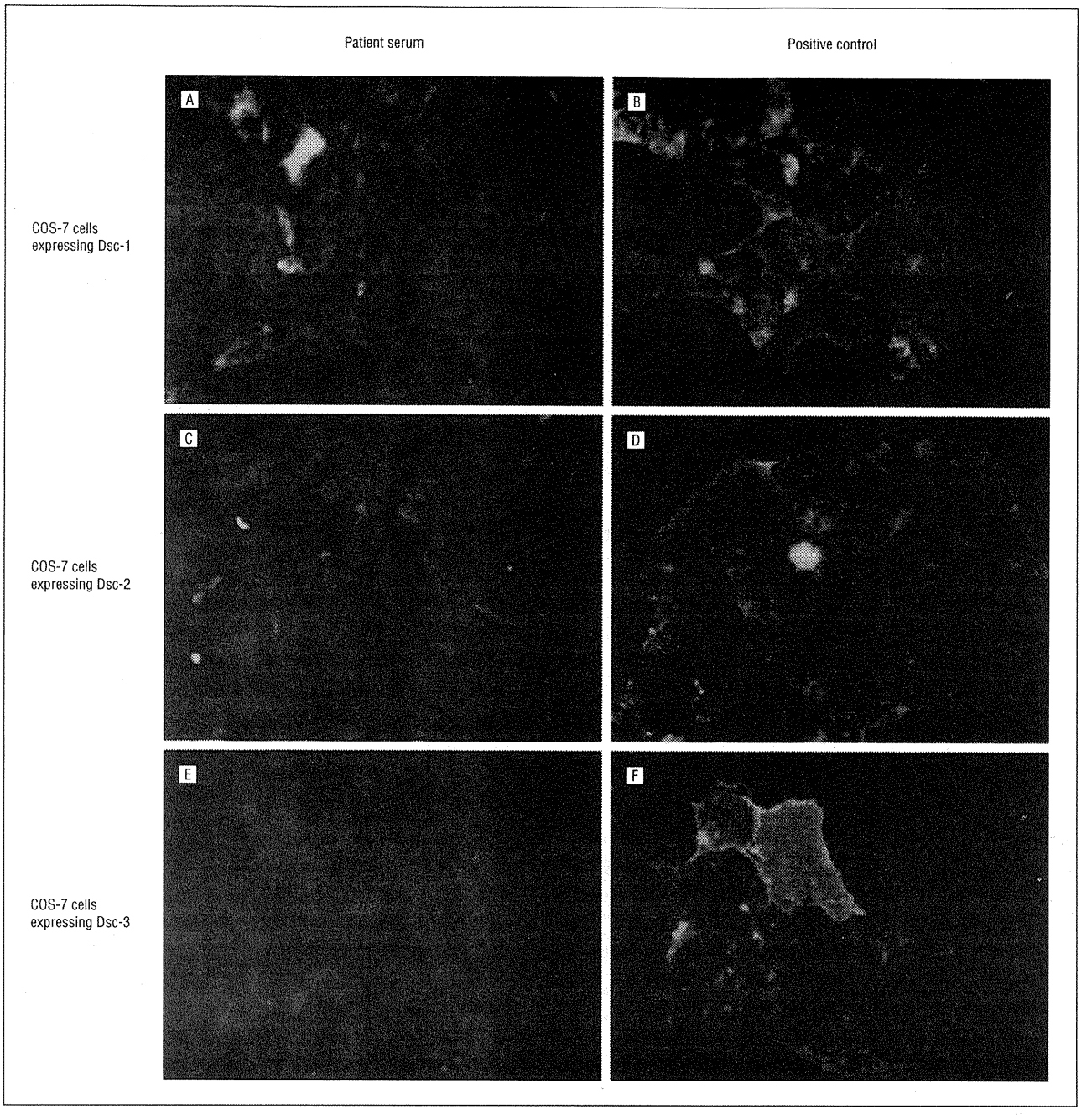

Figure 2. Immunofluorescence findings. Immunofluorescence of transfected COS-7 cells demonstrating no desmocollin 1 (Dsc1) (A), Dsc2 (C), or Dsc3 (E) staining in cells incubated with the patient's serum. Preparation of mammalian cell expression constructs of human Dsc1, Dsc2, and Dsc3 with transfection into COS-7 cells was performed as previously described.[13] Immunofluorescence of unfixed COS-7 cells was performed using the method by Stanley et al.[15] A control serum sample from a patient with atypical pemphigus known to have anti-Dsc1 (B), anti-Dsc2 (D), and anti-Dsc3 (F) was used as a positive control and demonstrated granular staining of the cell surface.

BP180, 160-kDa Dsg1, 210-kDa envoplakin, 190-kDa periplakin, and 110-kDa Dsc a-form and b-form antigens (Figure 3). Because of the patient's unexpected death and limited amount of serum available for studies, indirect IF on rat bladder was not performed.

COMMENT

The association of cancer with immunobullous diseases can be seen in patients with PNP and in patients with typical PV.[9-11] Paraneoplastic pemphigus is a specific diagnostic entity, first described in 1990 by Anhalt et al.[16] Most patients with PNP have anti-Dsg3 antibodies; however, a disease mechanism relying exclusively on anti-Dsg3 antibody is thought to be unlikely. Ohyama et al[17] demonstrated that the association between clinical phenotype and anti-Dsg autoantibody profile in PNP is not as clear as that in classic PV, and they suggested that the skin blisters in PNP can be caused not only by anti-Dsg antibodies but also by other pathologic mechanisms, such as a lichenoid reaction and interface dermatitis. In contrast, Amagai et al[18] showed that affinity-purified anti-

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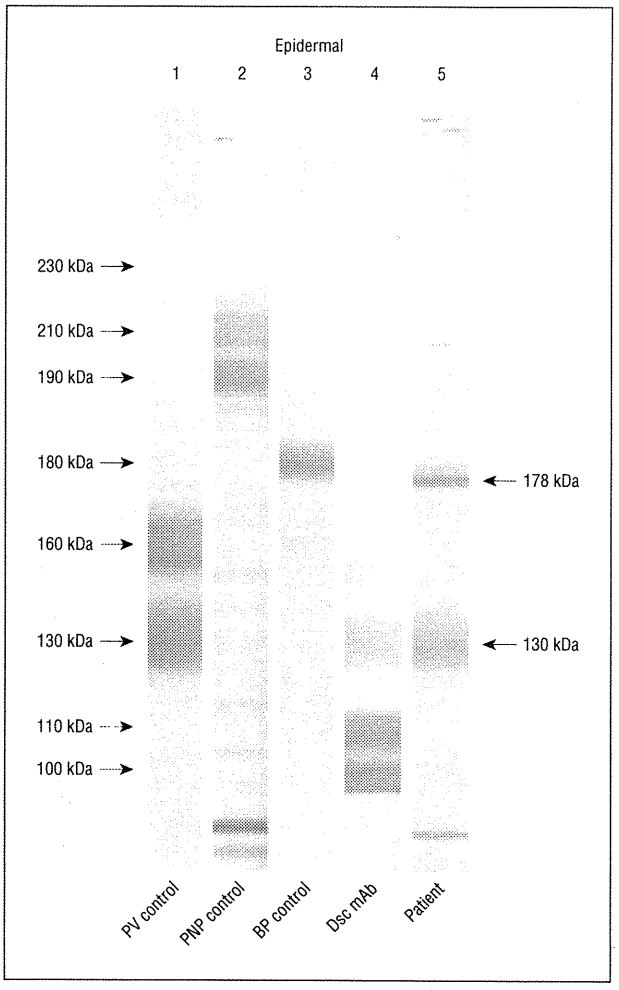

Figure 3. Immunoblotting using normal human epidermal extracts as the source of antigens demonstrating IgG autoantibodies against desmoglein 3 (Dsg3) 130-kDa antigen and against an unknown 178-kDa protein in the patient's serum (lane 5). Immunoblotting was negative for autoantibodies against bullous pemphigoid 230 (BP230), BP180, 160-kDa Dsg1, 210-kDa envoplakin, 190-kDa periplakin, and 110-kDa desmocollin a-form and b-form antigens. Serum samples from patients with pemphigus vulgaris (PV) (lane 1), paraneoplastic pemphigus (PNP) (lane 2), bullous pemphigoid (lane 3), and anti–desmocollin monoclonal antibodies (Dsc mAb) (lane 4) were used as positive control specimens.

Dsg3 from serum samples of patients with PNP caused gross blisters in neonatal mice and that the removal of anti-Dsg3 eliminated this ability, suggesting that anti-Dsg3 IgG has a primary pathogenic role in inducing loss of cell adhesion between keratinocytes and in causing blister formation. It is possible that much of the initial damage may be the result of anti-Dsg antibodies and that the polymorphous nature of the condition is likely caused by further interaction of antibodies directed against the plakin family of proteins.

Our patient had a negative ELISA for anti-BP230 antibodies, and immunoblotting was negative for the 210-kDa envoplakin and the 190-kDa periplakin. Her immunologic profile, lack of mucosal involvement, and excellent response to therapy are consistent with the diagnosis of PH and render the diagnosis of PNP unlikely. However, the association of a pulmonary malignant neoplasm with this unusual skin presentation may indicate that this patient in fact represents a case of paraneoplastic PH.

In contrast to PNP, the association of PH with neoplasia is rare, and only 6 cases have been reported in the literature to our knowledge, including 4 patients with lung cancer,[19-22] 1 patient with esophageal cancer,[23] and 1 patient with prostate cancer.[24] Because of the few cases and limited description, it is difficult to draw any conclusions regarding the immunologic profiles of these patients. No circulating antibodies were found in 1 case,[19] autoantibodies against 150-kDa and 230-kDa antigens were reported in 1 case,[20] low-titer IgG antibodies against cell surfaces of the epithelium of guinea pig esophagus were demonstrated in 1 case,[22] and detailed information was unavailable from another case report of a patient with lung neoplasia.[21] The patient with prostate cancer showed anti-Dsg1 and anti-Dsg3 antibodies,[24] and the specificity of circulating antibodies found on indirect IF in the patient with esophageal cancer was unidentified.[23] Among all cases, a strict parallel course between dermatosis and the malignant neoplasm was reported only by Palleschi and Giomi,[20] who suggested that some cases of PH may in fact represent a tumor-related cutaneous disease and that these may be included in a wider definition of PNP.

It is unclear why the clinical presentations of PH and classic pemphigus are so different, despite the common presence of anti-Dsg1 or anti-Dsg3 autoantibodies. It has been speculated that the phenotypic variations may be caused by differences in epitopes recognized by the autoantibodies. Whereas autoantibodies in pemphigus foliaceus and PV recognize functionally important regions on Dsg and inhibit their adhesive function, autoantibodies in PH may recognize a functionally less important part of the molecule, thereby inducing no acantholysis but inflammatory processes via complement activation or cytokine release by keratinocytes, leading to intercellular edema and eosinophilic spongiosis.[1,6,25,26] Changes in epitopes recognized by anti-Dsg autoantibodies may also explain the transformation of PH into pemphigus foliaceus or PV.[1,7,8] Few patients with PH demonstrate autoantibodies to Dsg3, and our patient's immunologic profile supports the concept that Dsg3 is also a target antigen in this disease.[1,3,6-8] Many of these cases, including our patient, did not have mucosal involvement, and this further reinforces that the pathogenesis of PH autoantibodies differs from that of classic pemphigus and that other unknown factors may be involved.

There is a growing body of evidence in the literature demonstrating the importance of Dsc3 in cellular adhesion of the epidermis.[27-30] It has recently been shown that many patients with PH have anti-Dsc autoantibodies (K. Ishii, MD, and TH, unpublished data, 2009). However, in our patient, 3 different methods (ELISA, immunoblotting, and IF of transfected COS-7 cells) failed to demonstrate IgG anti-Dsc1, anti-Dsc2, or anti-Dsc3 antibodies. However, immunoblot analysis in our patient demonstrated an unknown 178-kDa protein in the serum. Unfortunately, because of the patient's unexpected death, we were unable to further investigate the characteristics of this protein. It is unclear if the presence of these autoantibodies was associated with the unique clinical presentation of our patient with acantholysis or with the underlying neoplasm. Further case

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reports and investigation with detailed immunologic profiles are required to fully understand the underlying immunopathogenic nature of this disease.

Herein, we described a patient having PH with IgG autoantibodies to Dsg3 and to an unknown 178-kDa protein who had an underlying lung neoplasm. To our knowledge, this is the first report of PH with an associated neoplasm in which only anti-Dsg3 antibody was detected. It is unclear if other factors such as non-IgG autoantibodies or the anti–178-kDa protein were involved in our patient's unusual presentation.

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CASE REPORT

Mucous membrane pemphigoid with immunoglobulin G autoantibodies against full-length and 120-kDa ectodomain of BP180

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ABSTRACT

Mucous membrane pemphigoid (MMP) is a rare autoimmune, subepidermal, bullous disease characterized by erosive lesions on the mucous membranes and skin. MMP reacts with various target antigens including BP180, laminin-332, β4 integrin, α6 integrin or type VII collagen. We present a 67-year-old male MMP patient who had lesions on the oral and ocular mucous membranes and facial skin. By immunoblot analyses, immunoglobulin G autoantibodies in the patient's sera reacted with full-length BP180 and the 120-kDa ectodomain of BP180 (LAD-1).

Key words: bullous pemphigoid antigen 2, LAD-1, mucous membrane pemphigoid.

INTRODUCTION

Mucous membrane pemphigoid (MMP), previously referred to as cicatricial pemphigoid, is an acquired autoimmune blistering disease with erosive lesions involving primarily the oral and ocular mucous membranes that result in scarring.[1–3] The skin is involved in 25–35% of patients with MMP and the most frequently affected areas are the scalp, head and neck. Immunochemical techniques have identified laminin-332, BP180 (bullous pemphigoid antigen 2 or type XVII collagen) and the β4 integrin as the major targets of autoantibodies in MMP.[3,4]

Autoantibodies against BP180 are associated with bullous pemphigoid (BP), MMP and linear immunoglobulin (Ig)A dermatosis (LAD), all of which share several overlapping clinical features despite their different target epitopes and subclasses of immunoglobulin.[5] BP180 is a 180-kDa transmembrane protein with a non-collagenous intracellular component and a predominantly collagenous extracellular component.[6] The NC16a domain, which is the major epitope for BP, is in a small non-collagenous region of BP180 that is just outside the plasma membrane.[6] The autoantibodies from patients with MMP have been reported to recognize particular epitopes, the NC16a domain of BP180, the C-terminus of the ectodomain, or the soluble BP180 ectodomain (LAD-1).[1,3]

We report an MMP case in which immunoblot analysis demonstrated IgG autoantibodies reacted not only with full-length BP180 but also with LAD-1, which is the 120-kDa degradation product of the ectodomain of BP180 and the major antigen for IgA autoantibodies in LAD.[7]

CASE REPORT

A 67-year-old man presented with a 6-year history of erosions in the oral cavity and conjunctiva. In addition, he complained of progressive impairment of

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vision. Then, erosion had appeared on the penis but resolved spontaneously in a few months. He had no family history of any skin disorders.

A physical examination revealed erythematous erosions and white atrophic scars along the gingival mucosa and hard palate (Fig. 1a). The ocular mucous membrane exhibited conjunctival inflammation, symblepharon, neovascularization and early corneal scarring (Fig. 1b). Due to the presence of symblepharon, the severity of ocular involvement was graded as stage III according to the modified Mondino–Foster staging system.[8] There were atrophic scars on the face (Fig. 1c). The scalp also exhibited a few cicatricial alopecic patches. No lesions were observed on the anogenital area or the skin at any other body sites.

Direct immunofluorescence (IF) on the perilesional area of the gingiva was negative. However, indirect IF using normal human skin substrate revealed IgG anti-basement membrane zone (BMZ) antibodies at a titer of 1:160. On indirect IF using salt-split normal human skin, circulating IgG autoantibodies bound to the epidermal side of the split skin at a titer of 1:40 (Fig. 1d). Circulating IgA autoantibodies were not detected. To further characterize the antigen to which the patient's IgG bound, we performed an immunoblot analyses. The patient's serum IgG recognized BP180 and faintly BP230 by immunoblot analysis using normal human epidermal extract (Fig. 2a). IgA and IgG autoantibodies did not show any reactivity with either the NC16a domain recombinant protein or the C-terminal domain recombinant

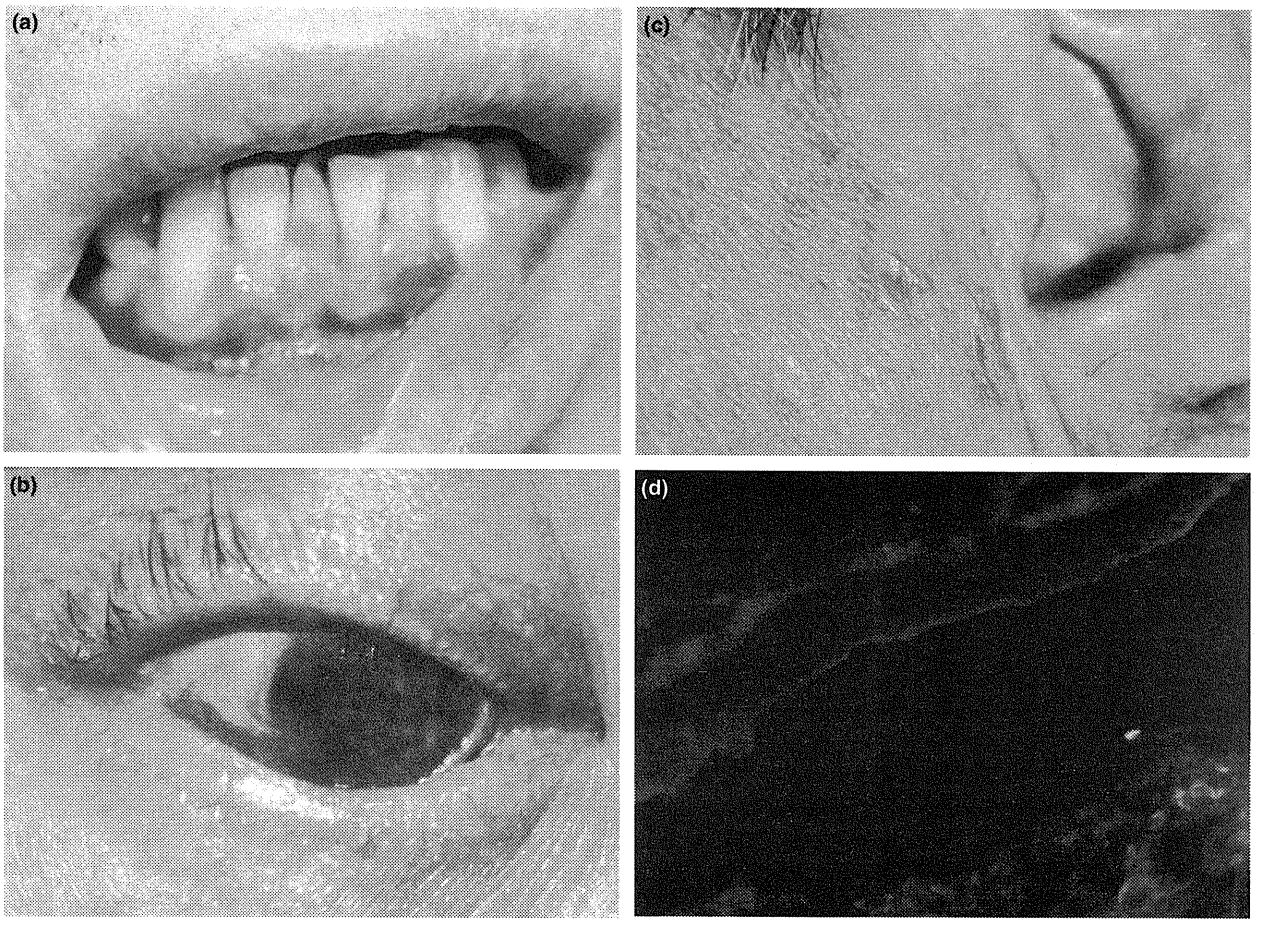

Figure 1. Clinical features of the patient. (a) Desquamative gingivitis along the lower gingival mucosa. (b) Ocular involvement with chronic conjunctivitis and symblepharon. (c) Atrophic scar on the face. (d) Indirect immunofluorescence using 1 mol/L salt-split skin revealed the presence of immunoglobulin G autoantibodies that bound to the epidermal side of salt-split skin.

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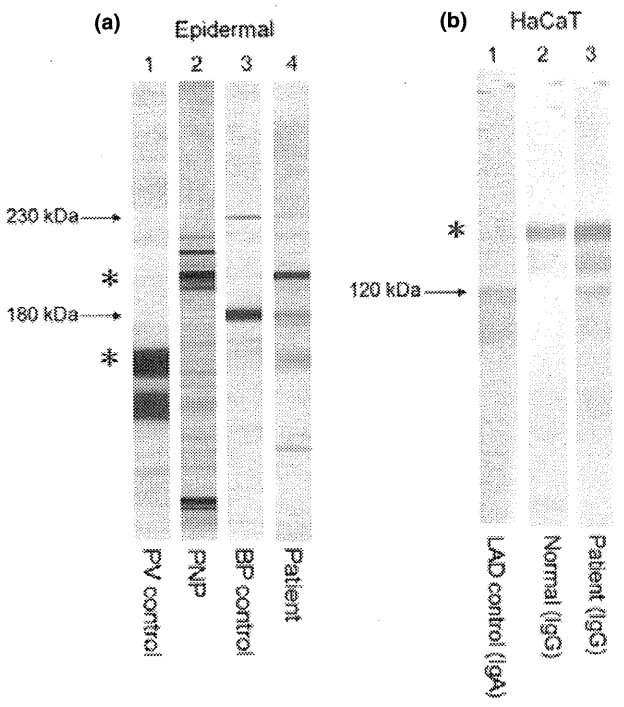

Figure 2. Immunoblot analyses. (a) Immunoblot analysis using normal human epidermal extracts. The patient's serum immunoglobulin (Ig)G reacted weakly with BP230 (upper arrow) and clearly with BP180 (lower arrow). This serum also reacted with two additional proteins which co-migrated with the 190-kDa periplakin (upper asterisk) and the 160-kDa desmoglein 1 (lower asterisk). However, these reactivities were considered non-specific, because the serum did not show any keratinocyte cell surface staining by indirect IF. (b) Immunoblot analysis using concentrated HaCaT cell culture supernatant revealed IgG autoantibodies reacting with the 120-kDa linear IgA dermatosis (LAD)-1 in the patient serum. The asterisk indicates non-specific reactivity. BP, bullous pemphigoid; PNP, paraneoplastic pemphigus; PV, pemphigus vulgaris.

protein by immunoblot analysis (data not shown). We also performed enzyme-linked immunosorbent assay (ELISA) using recombinant protein of BP180 NC16a and recombinant BP230, and the results for IgG antibodies were negative; ELISA of BP180 NC16a index was 2.46 (positive index ≥15) and ELISA of BP230 index was 2.55 (positive index ≥9). Therefore, we confirmed this patient had no autoantibody to NC16a domain of BP180 and BP230. Interestingly, IgG autoantibodies reacted with the 120-kDa LAD-1 by immunoblot analysis using concentrated HaCaT cell culture supernatant (Fig. 2b). IgG autoantibodies did not react with laminin-332 by immunoblot analysis using purified human laminin-332 (data not shown). These results indicated that our patient's IgG autoantibodies targeted antigenic sites on the soluble ectodomain of BP180, but did not react with the NC16a or C-terminal domain of BP180. These results suggested that the patient's IgG autoantibodies reacted with epitope(s) between the NC16a domain and the C-terminal domain in LAD-1.[9,10]

Based on the clinical and immunochemical findings, the patient was diagnosed as having MMP. Treatment initiated with oral methylprednisolone 16 mg, dapsone 50 mg and mycophenolate mofetil 2.0 g daily. Three months later, although the ocular lesion persisted, the patient's oral and skin lesions were much improved and remained in partial remission on the therapy.

DISCUSSION

Mucous membrane pemphigoid is a heterogeneous disease with respect to the clinical site of involvement (oral, ocular, nasopharyngeal, laryngeal, esophageal, anogenital and skin lesions), the isotype of autoantibodies involved (IgG, IgA or both), the autoantibody binding site by indirect IF on salt-split skin (epidermal, dermal or both) and the target antigens involved (BP180, BP230, β4 integrin, α6 integrin, laminin-332 and type VII collagen).[1–4]

We described a patient with MMP with lesions on the oral and ocular mucous membranes and the skin, and with circulating IgG autoantibodies binding to the epidermal side of salt-split skin. Most MMP patients with IgG antibodies bound to the epidermal side recognize the NC16a domain or the C-terminal domain of BP180.[3,10] However, the IgG autoantibodies of our patient did not react with the recombinant proteins of the NC16a domain or the C-terminal domain of BP180, but instead reacted with the whole molecule of BP180 and its 120-kDa soluble ectodomain LAD-1. The NC16a domain comprises amino acids 490–562 and the LAD-1 molecule includes amino acids 524–1497.[11] Therefore, the epitope(s) should be present in the central rod-like collagenous domain of BP180. Although the function of this domain has not been well characterized, this result may indicate the importance of this domain for interaction of BP180 with the epidermal basement zone.

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The NC16a domain and the C-terminal domain are well known to be immunodominant regions in MMP, while LAD-1 was initially described as a target antigen in LAD. Only a few MMP patients were reported to have autoantibodies to LAD-1 without autoantibodies to the NC16a or C-terminal fragments.[9] Schmidt *et al.*[9] demonstrated that sera that showed immunoreactivity with LAD-1 but not with NC16a or C-terminal fragments were found in four of 26 patients with MMP (15.4%). Moreover, autoantibodies against the NC16a domain were predominantly IgG, whereas LAD-1 was recognized mainly by IgA.[4] Thus, MMP patients with IgG autoantibodies to LAD-1 but not to either the NC16a domain or the C-terminal domain were considered to be rare. To our knowledge, this is the first report to describe clinical features in a patient with MMP demonstrating IgG autoantibodies against LAD-1 in the absence of autoantibodies against the NC16a domain or the C-terminal end of the BP180 ectodomain.

In a recent study on 124 patients with MMP, Oyama *et al.*[4] reported that patients with more severe clinical phenotypes had predominantly IgG and IgA reactivity to both full-length BP180 and LAD-1, whereas the autoantibody reactivity to just a single BP180-related antigen was associated with a milder clinical phenotype of MMP. Comparable to these findings, our patient also showed relatively severe disease activity, as he had erosions and scars on the oral mucosa, stage III ocular involvement and skin lesions. However, it remains unclear whether the development of multiple antigenic targets is of pathogenic significance in MMP or whether it is a secondary phenomenon during progression to more severe disease.[4] Future studies are needed to examine whether the multiple different autoantibodies are related to the different phenotypes and disease severity in MMP.

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Mucous membrane pemphigoid with autoantibodies to all the laminin 332 subunits and fatal outcome resulting from liver cirrhosis and hepatocellular carcinoma

To the Editor: Mucous membrane pemphigoid (MMP) patients rarely have autoantibodies to laminin 332 (formerly laminin 5).[1] In most cases, autoantibodies to the laminin $\alpha 3$ subunit are detected, although MMP patients with autoantibodies to the laminin $\gamma 2$ subunit alone have been reported.[2] However, MMP patients with autoantibodies to all three subunits of laminin 332 have not to our knowledge previously been reported.

A 73-year-old woman suffered from liver cirrhosis with hepatitis C virus infection for 17 years. She previously had undergone splenectomy. A hepatocellular carcinoma mass 2.5 cm in diameter persisted in the left lobule despite radiofrequency ablation and microwave coagulation therapy. A 9-year history of diabetes mellitus was currently managed with insulin injections. Three months before her visit, bullae had developed on her elbows and knees. A biopsy specimen from the left patellar skin showed subepidermal blister formation with a mild chronic inflammatory infiltrate in the papillary dermis, which is suggestive of bullous pemphigoid. Indirect immunofluorescence with the patient's serum demonstrated immunoglobulin G (IgG) antibodies to the basement membrane zone of healthy human skin sections. Indirect immunofluorescence with salt-split skin revealed IgG antibodies reactive with the dermal side (not shown). By immunoblotting using normal human dermal extract, the patient's serum reacted with neither the 290-kDa type VII collagen nor the 200-kDa laminin $\gamma 1$ subunit[3] (Fig 1, *left*). By immunoblotting using purified human laminin 332[4] as an antigen source, the patient's IgG antibodies reacted strongly with all the $\alpha 3$, $\beta 3$, and $\gamma 2$ subunits (Fig 1, *right*). We diagnosed this case as antilaminin 332 MMP.

Despite the administration of 200 mg of minocycline and 75 mg of dapsone daily together with a topical steroid, oral ulcers and blister formation spread rapidly over the body, together with the development of fever, hoarseness, and epistaxis. Oral prednisolone 30 mg daily had no effect and was tapered to 20 mg daily. Any additional steroids or immunosuppressive agents were avoided because of possible secondary infection. Advanced liver cirrhosis did not permit plasmapheresis. Intravenous immunoglobulin injection was not administered because of the poor prognosis of severe liver cirrhosis associated with cancer. Widespread painful erosions developed (Fig 2).

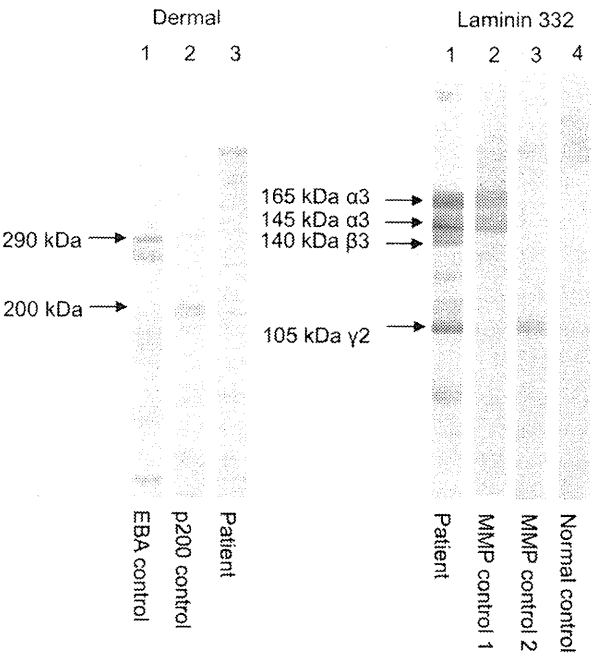

Fig 1. Immunoblot analyses. Using dermal extracts (*left column*), IgG autoantibodies of control epidermolysis bullosa acquisita serum reacted with the 290-kDa type VII collagen (lane 1), and immunoglobulin G (IgG) autoantibodies of control antilaminin $\gamma 1$ pemphigoid (formerly anti-p200 pemphigoid) serum reacted with 200-kDa laminin $\gamma 1$ (lane 2), whereas neither antigen was detected by our patient's serum (lane 3). Using purified human laminin 332 (*right column*), IgG antibodies of this patient's serum reacted with all the 165-kDa $\alpha 3$, 145-kDa $\alpha 3$, 140-kDa $\beta 3$, and 105-kDa $\gamma 2$ subunits of laminin 332 (lane 1). IgG antibodies of control MMP serum 1 reacted with the 165- and 145-kDa $\alpha 3$ subunits (lane 2), and IgG antibodies of control MMP serum 2 reacted with the 105-kDa $\gamma 2$ subunit (lane 3), while normal serum showed no reactivity (lane 4).

The patient was severely depressed and her mental and physical activities were rapidly lost. Portal vein thrombosis occurred, and the patient died of sepsis. Eye lesions were not apparent throughout her course.

To our knowledge, this is the first case of MMP with autoantibodies to all three subunits of laminin 332. This set of autoantibodies may be responsible for the extensive distribution and severity of disease in the present case. Antilaminin 332 MMP is associated with cancer, although hepatocellular carcinoma has never been reported.[5]

In an immunocompromised patient, extensive skin lesions can cause sepsis as a result of direct infection of the skin. Indirectly, however, widespread erosions can cause exacerbation of hypoalbuminemia and constipation following an extended

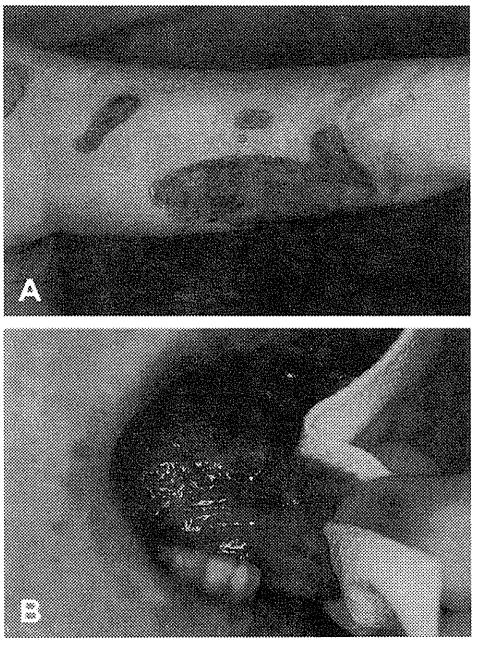

Fig 2. Clinical manifestations in the terminal stage of the present case. **A**, Fresh, large, well demarcated erosions without perilesional erythema on the left upper arm. **B**, Ulcers on the oral mucosa and tongue.

bedridden state; both can trigger portal vein thrombosis followed by spontaneous bacterial peritonitis[6] and sepsis, although a bacterial culture of ascitic fluid was not performed in the present case. Caregivers should pay attention to any infection, including spontaneous bacterial peritonitis, during the course of a severe bullous disease in immunocompromised patients with liver cirrhosis.

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Cancrum oris in a boy with Down syndrome

To the Editor: A 17-year-old Italian male with Down syndrome presented to the dermatology department with a 2-cm ulcerative lesion on the right tip of his tongue (Fig 1). The onset was about 7 days earlier with mild swelling. The patient's parents described a persistent vesicular eruption in the same area during the previous 2 months. They attributed it to biting and self-inflicted injuries and treated it with multiple but inefficacious drugs (nystatin, metisoprinol, diflucortolone, and chloramphenicol). The physical examination revealed that the right submandibular lymph nodes were slightly enlarged and tender. Fever and malaise were not reported, and neither were any other symptoms. Routine hematologic parameters were normal, apart from a chronic low leukocyte count. These findings were similar to his previous laboratory examinations. His medical history, as reported by the parents, was negative for any kind of recurrent infection. He was otherwise in good health and had never been institutionalized. An HIV test was negative. An oral swab revealed significant growth of multiple anaerobic bacteria. Cancrum oris was strongly suspected, and a 3-mm punch biopsy of the lesion was performed. Histologic examination revealed a nonspecific acute process characterized by a heavy inflammatory infiltrate mainly comprised of neutrophilic abscesses and blood vessel endothelial swelling, suggestive of bacterial infection. As indicated by microorganism culture and sensitivity tests, an intramuscular dose of lyncomicin 600 mg (2 mL)

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Development of an ELISA for sensitive and specific detection of IgA autoantibodies against BP180 in pemphigoid diseases

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Abstract

Background: Pemphigoids are rare diseases associated with IgG, IgE and IgA autoantibodies against collagen XVII/BP180. An entity of the pemphigoid group is the lamina lucida-type of linear IgA disease (IgA pemphigoid) characterized by IgA autoantibodies against BP180. While for the detection of IgG and IgE autoantibodies specific to collagen XVII several ELISA systems have been established, no quantitative immunoassay has been yet developed for IgA autoantibodies. Therefore, the aim of the present study was to develop an ELISA to detect IgA autoantibodies against collagen XVII in the sera of patients with pemphigoids.

Methods: We expressed a soluble recombinant form of the collagen XVII ectodomain in mammalian cells. Reactivity of IgA autoantibodies from patients with IgA pemphigoid was assessed by immunofluorescence microscopy and immunoblot analysis. ELISA test conditions were determined by chessboard titration experiments. The sensitivity, specificity and the cut-off were determined by receiver-operating characteristics analysis.

Results: The optimized assay was carried out using sera from patients with IgA pemphigoid (n = 30) and healthy donors (n = 105). By receiver operating characteristics (ROC) analysis, an area under the curve of 0.993 was calculated, indicating an excellent discriminatory capacity. Thus, a sensitivity and specificity of 83.3% and 100%, respectively, was determined for a cut-off point of 0.48. As additional control groups, sera from patients with bullous pemphigoid (n = 31) and dermatitis herpetiformis (n = 50), a disease associated with IgA autoantibodies against epidermal transglutaminase, were tested. In 26% of bullous pemphigoid patients, IgA autoantibodies recognized the ectodomain of collagen XVII. One of 50 (2%) of dermatitis herpetiformis patients sera slightly topped the cut-off value.

Conclusions: We developed the first ELISA for the specific and sensitive detection of serum IgA autoantibodies specific to collagen XVII in patients with pemphigoids. This immunoassay should prove a useful tool for clinical and translational research and should essentially improve the diagnosis and disease monitoring of patients with IgA pemphigoid. Moreover, our findings strongly suggest that IgA pemphigoid and IgG bullous pemphigoid represent two ends of the clinical spectrum of an immunological loss of tolerance against components of hemidesmosomes, which is mediated by both IgG and IgA autoantibodies.

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Background

Pemphigoids are rare autoimmune blistering disorders associated with autoimmunity against hemidesmosomal proteins [1]. Main entities of the pemphigoid group include bullous pemphigoid, pemphigoid gestationis, linear IgA disease, mucous membrane pemphigoid and lichen planus pemphigoides with an approximate annual incidence of 7, 0.5, 0.5, 1 and undefined cases in one million, respectively [2-5]. A major target of pemphigoid autoantibodies is the bullous pemphigoid antigen of 180 kDa (BP180), also referred to as collagen XVII, a hemidesmosomal transmembrane protein with a type II orientation whose extracellular domain consists of 15 collagenous regions interrupted by non-collagenous portions (Figure 1A) [1,4,6]. In a minority of patients, IgA reactivity against BP230, an intracellular hemidesmosomal component, has been detected [7]. A hallmark of collagen XVII is its constitutive shedding yielding a shorter and soluble form of the molecule that spans most of its ectodomain [8,9].

BP180 is targeted by autoantibodies of different Ig isotypes, including different IgG subclasses, IgA and IgE [10-13]. The pathogenic relevance of IgG autoantibodies against BP180 is supported by several lines of evidence: 1) the transplacental transfer of pemphigoid IgG autoantibodies from mothers to the fetus induces transient skin blistering in the newborn [14-16]; 2) serum levels of IgG autoantibodies against BP180 correlate with disease activity in patients with bullous pemphigoid and pemphigoid gestationis [17-20]; 3) patients autoantibodies against BP180 recruit leukocytes to the dermal-epidermal junction and induce dermal-epidermal separation of human skin [21,22]; 4) IgG antibodies against BP180 induce subepidermal blistering when passively transferred into neonatal autoantigen humanized, wild type mice and hamsters [23-26]; 5) grafting of human BP180 transgenic mouse skin induces an autoimmune response resulting in subepidermal blistering in wild-type animals [27,28]. IgE autoantibodies against BP180 correlate with disease activity in pemphigoid patients and induce eosinophil infiltration and dermal-epidermal separation when injected into human skin grafted on immunodeficient mice [29-31].

While the pathogenic potential of IgG and IgE autoantibodies against BP180 was characterized *ex vivo* and in animal models, the pathogenicity of IgA autoantibodies was relatively less studied [32]. Very recently, we demonstrated that IgA autoantibodies from patients with linear IgA disease induce granulocyte-dependent dermal-epidermal separation in cryosections of human skin (van der Steen et al, unpublished).

Linear IgA disease was defined as a new entity different from dermatitis herpetiformis on the basis of a linear IgA deposition at the dermal-epidermal junction [33,34]. Further studies revealed heterogeneous molecular specificity of the IgA autoantibodies in patients with linear IgA disease, including BP180, BP230, collagen VII as well as still unidentified antigens of 180-, 200-, and 285-kDa [35]. While in most patients, IgA autoantibodies bind to the epidermal side of the salt-split skin by indirect immunofluorescence (IF) microscopy, staining of the dermal side of the artificial split may be also detected. IgA autoantibodies from pemphigoid patient sera recognize several forms of BP180 and preferentially bind to proteolytic products of this autoantigen [36-38]. Initial studies have shown that a 97 kDa protein (LABD97) is responsible for basement membrane binding of IgA in patients with linear IgA disease and that this protein is recognized in human skin extracts by IgA autoantibodies, when immunoblot analysis is performed [36]. Further studies have shown that a 120 kDa protein, referred to as linear IgA disease antigen (LAD)-1, secreted into the supernatants of cultured keratinocytes is also target of linear IgA disease autoantibodies [37]. Based on antigenic cross-reactivity between LAD-1

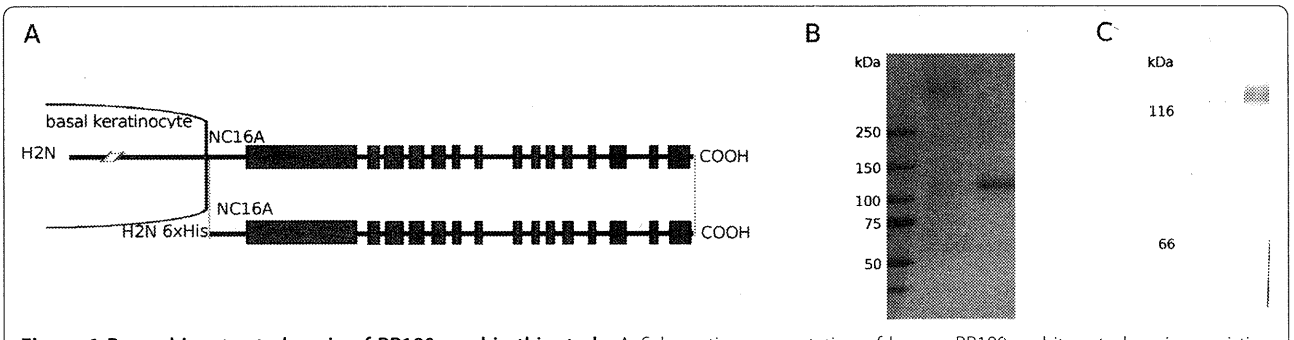

Figure 1 Recombinant ectodomain of BP180 used in this study. A: Schematic representation of human BP180 and its ectodomain consisting of alternating collagenous (C) and non-collagenous (NC) domains. The recombinant form of BP180 ectodomain used for the development of the current ELISA system spans from aa 490 through aa 1497 and has an NH_2-terminal hexahistidine tag. B: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the purified recombinant protein without and with previous boiling, which migrates around 360 (lane 2) and 120 kDa (lane 3), respectively. C: Immunoblot analysis of the native, normal human keratinocyte-derived shed BP180 ectodomain (lane 1) and the recombinant BP180 ectodomain (lane 2) using a monoclonal antibody specific to BP180. Weight markers of 116 and 66 kDa are shown to the left.