

CYTOTOXIC AND ANTIGEN PRESENTING CELLS, AND NON-BASEMENT MEMBRANE ZONE PATHOLOGY IN A CASE OF BULLOUS PEMPHIGOID

KOMÓRKI CYTOTOKSYCZNE I PREZENTUJĄCE ANTYPYGEN ORAZ PATOLOGIA STREFY POZA GRANICĄ SKÓRNO-NASKÓRKOWĄ NA PRZYKŁADZIE PEMPHIGOIDU PĘCHERZOWEGO

Ana Maria Abreu Velez¹, Vickie M. Brown², Michael S. Howard¹

¹Georgia Dermatopathology Associates, Atlanta, Georgia, USA

²Family Dermatology, Milledgeville, Georgia, USA

Corresponding author: Ana Maria Abreu-Velez, MD PhD abreuvelez@yahoo.com

Our Dermatol Online. 2012; 3(2): 93-99

Date of submission: 28.08.2011 / acceptance: 17.02.2012

Conflicts of interest: None

Abstract

Background: When performing direct and indirect skin immunofluorescence (DIF, IIF) in bullous pemphigoid (BP) utilizing single fluorophores such as fluorescein isothiocyanate (FITC), the presence of "background" fluorescence is commonly described. **Aim of reporting this case:** Our laboratory has noted that what is termed "background" could represent a complex immune response in BP, that may be present in addition to the classical deposits of immunoglobulins and/or complement at the dermal/epidermal basement membrane zone (BMZ). Therefore, we simultaneously used multiple colored fluorophores to further investigate this possibility. **Case Report:** A 68-year-old male was evaluated for the presence of rapidly appearing vesicles and bullae on the chest, with additional pruritus. **Methods:** Skin biopsies for hematoxylin and eosin (H&E) staining, as well as for DIF, IIF, salt split skin and immunohistochemistry (IHC) analyses were performed. **Results:** H&E staining demonstrated a subepidermal blistering disorder. Within the dermis, a mild, superficial and deep, perivascular infiltrate of lymphocytes, histiocytes and eosinophils was observed. A few infiltrate cells displayed positive DIF staining for CD5, CD8 and CD45 around dermal blood vessels, nerves and eccrine sweat glands. In contradistinction, CD4, CD56 and Granzyme B staining was predominantly negative in these areas. DIF, IIF and salt split skin studies revealed a strong presence of IgG, Complement/C3, IgM and fibrinogen in linear patterns at the BMZ. Around the upper dermal perivascular infiltrate, HAM56 and CD68 positive cells were also noted. Positive DIF staining for CD1a was found suprajacent to the blister in the epidermis. Utilizing identical antibodies, we repeated our staining with the IHC technique, to address the issue of autofluorescence in DIF staining. Our IHC findings correlated with DIF and IIF results. **Conclusion:** Using multiple DIF fluorophores in this case of BP, we observed autoantibodies to structures such dermal nerves, blood vessels and eccrine sweat glands, that were not appreciated using FITC alone. We propose the use of this technique in autoimmune skin diseases. In addition, we observed a prominent T cytotoxic cell infiltrate, that warrants further characterization.

Streszczenie

Wstęp: Podczas wykonywania bezpośredniej i pośredniej immunofluorescencji skóry (DIF, IIF) w pemfigoidzie (BP) wykorzystuje się pojedyncze fluorofory takie jak izotiocyanian fluoresceiny (FITC), a obecność „tła” fluorescencji jest powszechnie opisane. **Cel niniejszego raportu:** Nasze laboratorium zanotowało, że to, co jest określane jako „tło” może stanowić kompleksową odpowiedź immunologiczną w BP, która może być obecna dodatkowo w postaci klasycznych depozytów immunoglobulin i/lub dopełniacza w skórze/naskórku strefie błony podstawnej (BMZ). Dlatego równocześnie stosowaliśmy wiele kolorów fluoroforów do dalszego zbadania tej możliwości. **Opis przypadku:** 68-letni mężczyzna był oceniany pod kątem obecności szybko pojawiających się pęcherzyków i pęcherzy na piersi, z dodatkowym świądem. **Metody:** Przeprowadzono biopsję skóry z barwieniem hematoksyliną i eozyną (H&E), a także DIF, IIF, split skóry i immunohistochemiczną (IHC) analizę. **Wyniki:** Barwienie H&E wykazało subepidermalne pęcherze. W obrębie skóry właściwej obserwowano łagodne, powierzchowne i głębokie, okołonaczyniowe nacieki z limfocytów, histiocytów i eozynofili. Kilka nacieków komórkowych wykazywało pozytywne zabarwienie DIF dla CD5, CD8 i CD45 wokół skórnych naczyń krwionośnych, nerwów i ekrynowych gruczołów potowych. W przeciwieństwie do tych badań, barwienia CD4, CD56 i Granzym B było przeważnie ujemne w tych obszarach. DIF, IIF i badanie splitu skórno wykazały silną obecność IgG, komplement/C3, IgM i fibrynogenu w liniowych wzorach na BMZ. Pozytywne komórki zauważono również wokół górnych nacieków okołonaczyniowych w skórze, HAM56 i CD68. W bezpośrednio leżącym pęcherzu w naskórku stwierdzono pozytywne barwienie DIF dla CD1a. Wykorzystując identyfikację przeciwciał, powtarzaliśmy nasze barwienia techniką IHC, aby zająć się kwestią autofluorescencji w barwieniu DIF. Nasze badania IHC skorelowano z wynikami DIF i IIF. **Wnioski:** Wykorzystując wiele fluoroforów w DIF w tym przypadku BP, obserwowaliśmy autoprzeciwciała do takich struktur jak skórne nerwy, naczynia krwionośne i ekrynne gruczoły potowych, które nie zostały określone za pomocą samego FITC. Proponujemy wykorzystanie tej techniki w autoimmunologicznych chorobach skóry. Ponadto zaobserwowaliśmy znaczącą T cytotoksyczność komórek naciekowych, co wymaga dalszej charakterystyki.

Key words: bullous pemphigoid; cytotoxic T cells; nerves; blood vessels; sweat glands; mast cell tryptase

Słowa kluczowe: pemphigoid pęcherzowy; komórki T cytotoksyczne; naczynia krwionośne; gruczoły potowe; komórki tuczne; tryptaza

Abbreviations and acronyms: Bullous pemphigoid (BP), immunohistochemistry (IHC), direct and indirect immunofluorescence (DIF, IIF), hematoxylin and eosin (H&E), basement membrane zone (BMZ), mast cell tryptase (MCT).

Introduction

Current theory maintains that the development of skin lesions in bullous pemphigoid (BP) results from destruction of components of the basement membrane zone (BMZ) within the dermal-epidermal junction, secondary to autoantibodies deposited at the BMZ [1,2]. Two glycoproteins of molecular weight 230 kD (BPAG1) and 180 kD (BPAG2) serve as primary autoantigens in BP [1-4]. In BP, histologic dermal perivascular inflammatory infiltrates containing lymphocytes and eosinophils are classically appreciated, and linear IgG and complement/C3 deposits observed along the BMZ of the dermal-epidermal junction [1-4].

Case report

A 68-year-old male was evaluated for a two day duration of pruritic blisters on the chest. On physical examination, the chest displayed tense vesicles and bullae, with mild erythema at the lesional bases. A lesional skin biopsy was taken for hematoxylin and eosin (H&E) analysis. Biopsies for direct immunofluorescence and immunohistochemistry (DIF, IHC) studies were taken from the edge of the blistering area. Serum for indirect immunofluorescence (IIF) and salt split skin studies was also obtained [1].

DIF, and IIF on salt split skin: Our DIF and IIF were prepared and incubated with multiple fluorochromes, as previously described [1, 2, 4-13].

IHC: Performed as previously described [4-13]. For the IHC we utilized antibodies to IgG, IgA, IgM, IgD, IgE, Complement/C1q, Complement/C3c, Complement/C3d, anti-fibrinogen, anti-albumin, anti-kappa light chains, anti-lambda light chains, anti-CD1a, CD4, CD5, CD8, CD45, CD56, CD68, S100, mast cell tryptase (MCT), alpha 1 anti-trypsin, metalloproteinase matrix 9 (MMP9), linker of activated T cells (LAT), Zeta-chain-associated protein kinase 70 (ZAP-70) and ribonucleoprotein protein (RNP).

Results

Microscopic description:

Examination of the H&E tissue sections demonstrated a subepidermal blistering disorder. Within the blister lumen, numerous eosinophils were present, with

occasional lymphocytes also seen. Neutrophils were rare. Dermal papillary festoons were not observed. Within the dermis, a mild, superficial, perivascular infiltrate was noted, with additional mild, deep infiltrates around nerves and eccrine sweat glands. The dermal infiltrate contained lymphocytes, histiocytes and eosinophils. A PAS special stain showed reinforcement of the basement membrane zone (BMZ) of the dermal-epidermal junction, and no fungal organisms. DIF studies were performed utilizing simultaneous multiple antibody/multiple fluorochrome techniques, and revealed the following results: IgG (++, linear BMZ (salt split skin IIF demonstrated IgG on blister roof)1; IgA (-); IgM (+, linear band under the BMZ); IgG/M/A (++, linear at BMZ and on dermal eccrine glands and deep nerves); IgD(+/-, epidermal keratinocyte intracellular); IgE (-); Complement/C1q (-); Complement/C3 (+++), linear BMZ and on dermal eccrine glands); kappa light chains (++, linear BMZ and on dermal eccrine glands); lambda light chains (++, linear BMZ and on dermal eccrine glands); albumin (++, on dermal eccrine glands and deep nerves) and fibrinogen (++, linear BMZ, on dermal eccrine glands and deep nerves) (Fig. 1-3). The IHC studies showed a few infiltrate cells with positive staining for CD5, CD8 and CD45 around dermal blood vessels, nerves and eccrine sweat glands. In contradistinction, CD4, CD56 and Granzyme B staining was predominantly negative in these areas. DIF, IIF and salt split skin studies revealed a strong presence of IgG, Complement/C3, IgM and fibrinogen in linear patterns at the BMZ. Around the upper dermal perivascular infiltrate, positive IHC staining for HAM56 and CD68 was noted. Positive staining for CD1a was found suprajacent to the blister in the epidermis. (Fig. 1-3). Finally, p53 antibody was positive on a few cells in the epidermis above the blister. Mast cell tryptase (MCT) was strongly positive around most of the upper dermal blood vessels, where the primary dermal inflammatory process was seen. By using multiple fluorophores, in this case of BP we observed autoantibodies to structures such dermal nerves, blood vessels and eccrine sweat glands, that were not appreciated utilizing FITCI alone. Thus, we propose the use of this technique in autoimmune skin disease workup. In addition, we observed a predominant T cytotoxic cell infiltrate that warrants further characterization.

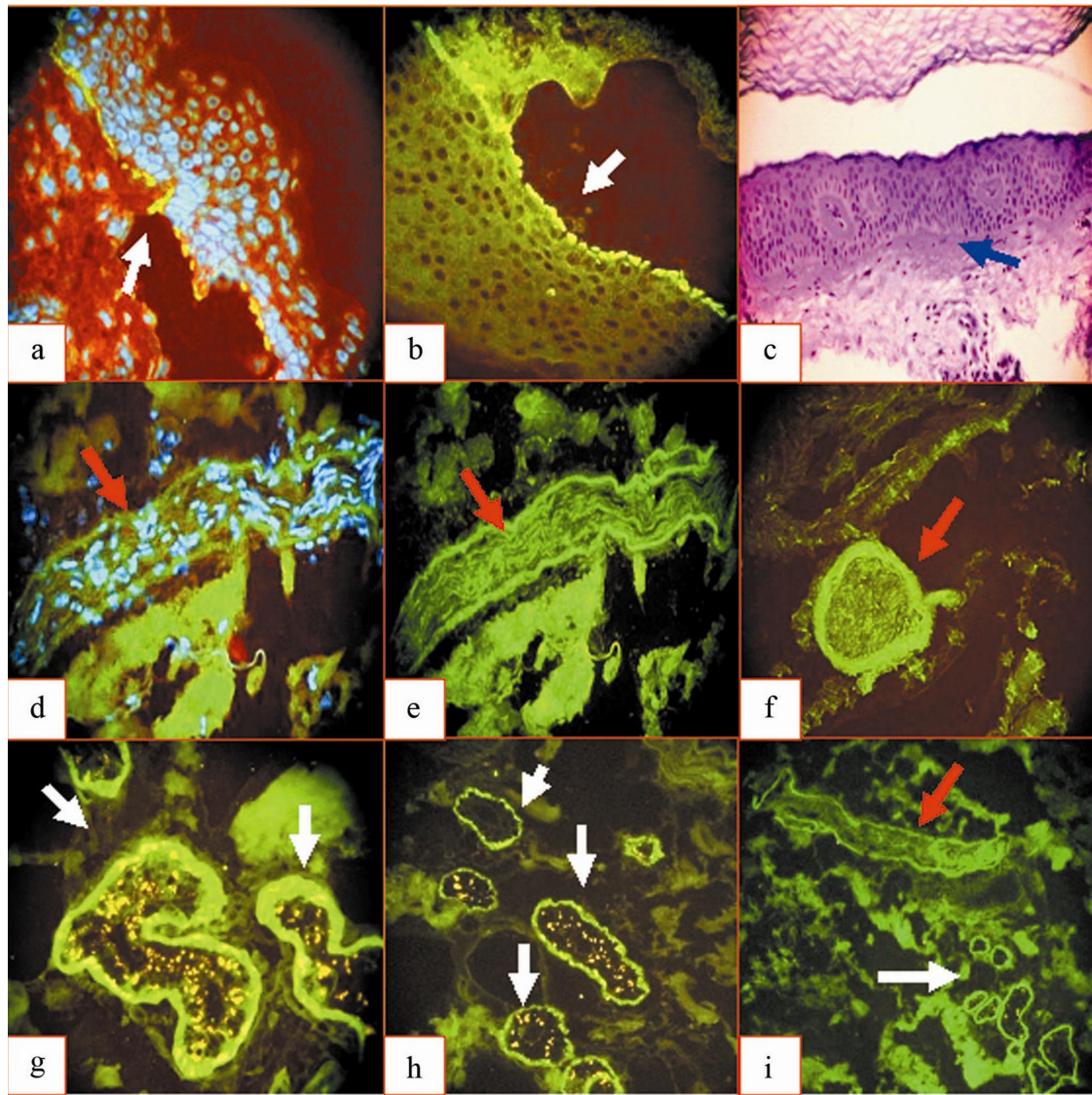


Figure 1. **a.** Positive BMZ staining utilizing 0.1 M sodium chloride salt split skin and indirect immunofluorescence (IIF). Note the positive staining on the upper/blister roof inner surface of the blister, using FITC conjugated anti-human complement/C3 (white arrow, yellow/green staining). The nuclei of the epidermal keratinocytes are counterstained with Dapi (light blue). **b.** Positive BMZ staining utilizing 0.1 M sodium chloride salt split skin and IIF. Note the positive staining on the lower/blister floor inner surface of the blister using FITC conjugated anti-human lambda light chain antibodies (white arrow, green staining). **c.** Positive PAS staining under the BMZ (blue arrow, red staining). **d.** Positive staining on a nerve with FITC conjugated anti-human IgG antibodies (red arrow, green staining). The neural cell nuclei are counterstained with Dapi (blue). **e.** Positive staining on a nerve utilizing FITC conjugated anti-human-IgG (red arrow, green staining). **f.** Positive staining on a nerve, using FITC conjugated anti-human fibrinogen (red arrow, green staining). **g.** Positive eccrine sweat gland staining with FITC conjugated anti-human-IgG (white arrows, yellow/green staining). **h.** Positive eccrine sweat gland staining utilizing FITC conjugated anti-human complement/C3 (white arrows, green staining). **i.** Simultaneous positive staining of a large, deep dermal nerve (red arrow) and a nearby eccrine sweat gland (white arrow) utilizing FITC conjugated anti-human-IgG (green staining).

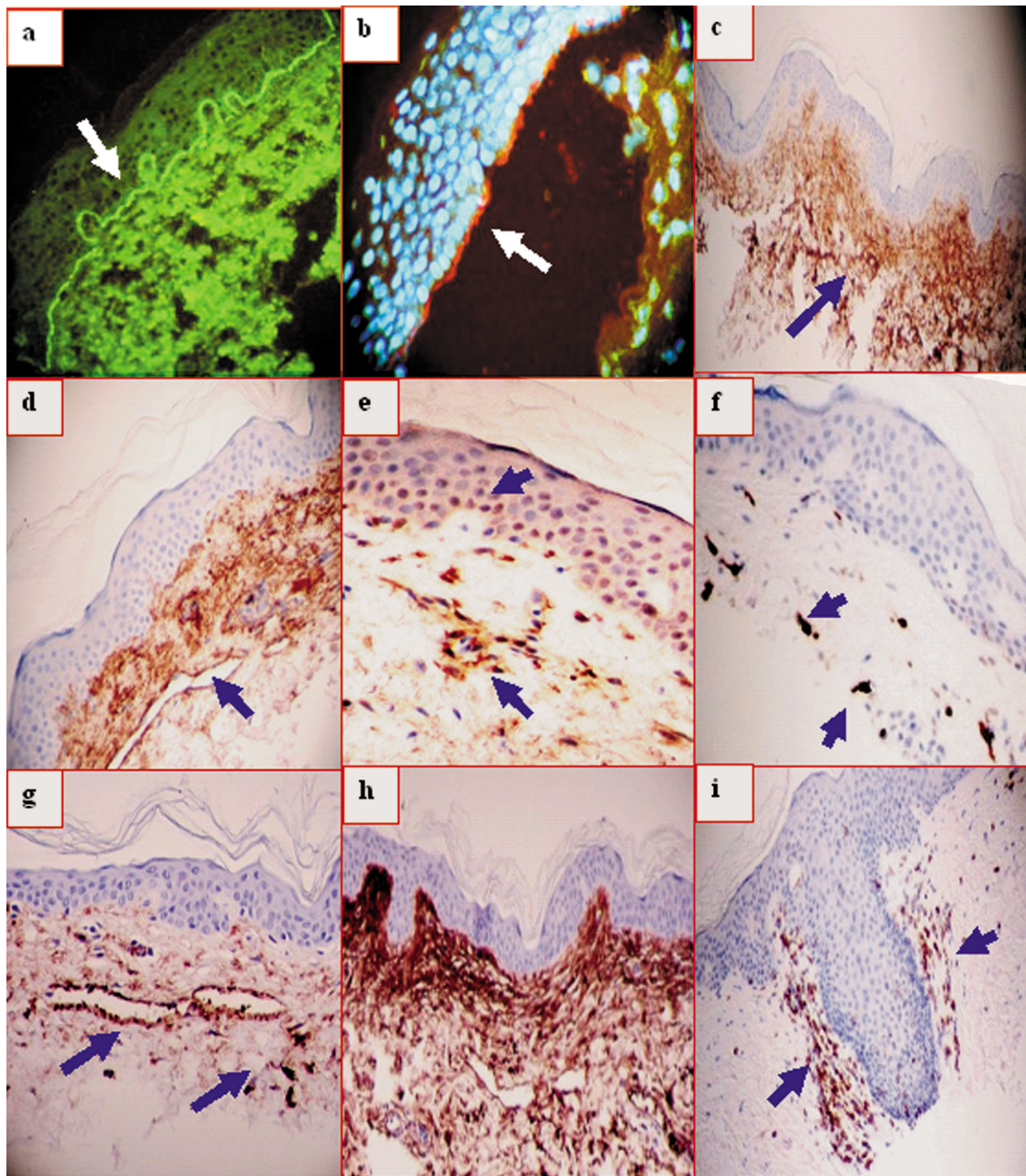


Figure 2. **a.** Positive linear staining on the BMZ utilizing FITC conjugated anti-human lambda light chain antibodies (white arrow, green staining). **b.** Positive linear staining on the BMZ utilizing Alexa 647 conjugated anti-human IgG (white arrow, red staining). **c.** Positive IHC staining for anti-human IgM in a pattern suggestive of dermal compartmentalization below under the BMZ (blue arrow, brown staining). **d.** Similar to c, but in this image, some upper dermal blood vessels are also positive for IgM (blue arrow, brown staining). **e.** Positive IHC staining with anti-human-IgE at the BMZ and on upper dermal blood vessels (blue arrows, brown staining). **f.** Positive IHC staining with mast cell Tryptase (MCT) around the upper dermal blood vessels (blue arrows, brown staining). **g.** Positive IHC staining with anti-human fibrinogen antibodies on upper dermal blood vessels (blue arrows, brown staining). **h.** Compartmentalization of IHC staining as a broad band under the BMZ with anti-human fibrinogen antibodies. Please note that the upper dermal blood vessels are also positive (brown staining). **i.** Positive IHC staining on a sub-epidermal cell infiltrate with CD5 antibodies (blue arrows, brown staining).

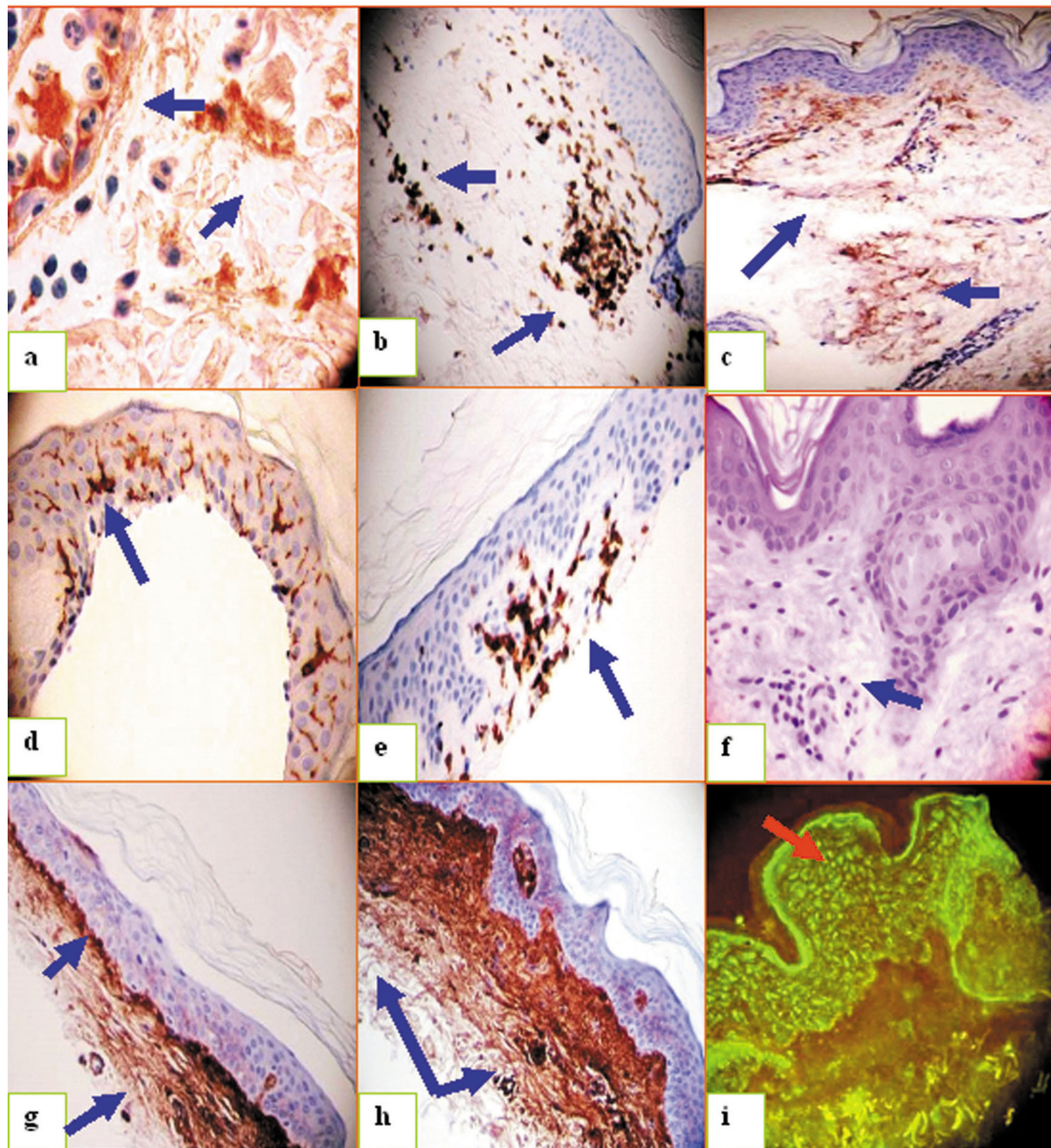


Figure 3. **a.** Positive IHC staining for anti-human albumin antibody, deposited on upper dermal blood vessels and small capillaries (blue arrows, brown staining). **b.** Positive IHC staining for CD45, on cells below the BMZ and surrounding upper dermal blood vessels (blue arrows, brown staining). **c.** Compartmentalization of IHC staining for Complement/C1q under the BMZ, and also involving upper dermal blood vessels and an eccrine gland ductus (blue arrows, brown staining). **d.** Positive IHC staining on Langerhans cells for CD1a, located within the epidermal stratum spinosum suprajacent to a bullous pemphigoid blister (blue arrow, brown staining). **e.** Positive IHC staining for CD3, on cells under the BMZ in the superficial dermis (blue arrow, brown staining). **f.** Eosinophils are noted on an H&E image, located within a perivascular upper dermal infiltrate (blue arrow). **g.** Positive IHC staining for Complement/C3 antibodies, present in a linear band along the BMZ and under the BMZ in a compartmentalized pattern in the upper dermis (blue arrows, brown staining). **h.** Note a similar IHC staining phenomenon as in g, but in this case using antibodies directed against human albumin (blue arrows, brown staining). **i.** Direct immunofluorescence (DIF) staining for FITC conjugated IgD; note the positive, punctate staining present within the epidermal stratum spinosum (red arrow, yellow/green staining).

Discussion

Classic research regarding immunoreactivity in BP has focused primarily on reactivity against the BMZ. Multiple animal models have been utilized to study this disorder, including both active and passive forms [3,4,12]. Recently, some studies have focused not only on damage to the BMZ of the skin, but also on damage associated with dermal blood vessels and nerves. Recently, one study suggested that cardiovascular events and thromboembolic diseases are important causes of death in patients with BP; the risk of stroke after a diagnosis of BP (relative to the general population) was investigated in Taiwan. The study sample included 390 patients with BP, versus 1950 matched subjects in a comparison group [14]. Other authors have also reported statistically significant cardiovascular and neurologic alterations between patients affected by BP and matched control groups [14-18].

As we have previously noted, most classic skin immunofluorescence studies have been performed with monofluorochrome techniques, frequently utilizing FITC. Utilizing this technique, additional FITC staining in areas other than the dermal-epidermal junction was disregarded as insignificant, background autofluorescence.

With the improvement of IHC techniques that allow differentiation between autofluorescence and genuine, diagnostic fluorescence [19,20], these previous assumptions regarding BP autofluorescence are under reconsideration.

In regard to our observed reactivity against dermal eccrine sweat glands and blood vessels, these structures are rich in integrins and other possible antigenic candidates. Soluble E-selectin (sE-selectin) represents an isoform of cell membrane E-selectin, an adhesion molecule synthesized only by endothelial cells. Soluble E-selectin has been reported to be significantly increased in the sera of the patients with BP [21-23]. One of the endothelial sE-selectin inducers is tumor necrosis factor- α (TNF- α), which is also able to enhance vascular endothelial growth factor (VEGF), a potent endothelium activator [21-23]. Thus, based on these reports and on our data, we suggest that further studies addressing BP autoreactivities in dermal sweat glands, nerves and blood vessels are warranted.

REFERENCES

1. Jordon RE., Beutner, EH, Witebsky E, Blumental G, Hale WL, Lever WF: Basement zone antibodies in bullous pemphigoid. *JAMA*. 1967; 200: 751-756.
2. Ghohestani RF, Nicolas JF, Rousselle P, Claudy AL: Diagnostic value of indirect immunofluorescence on sodium chloride-split skin in the differential diagnosis of subepidermal autoimmune blistering dermatoses. *Arch Dermatol*. 1997; 133: 1102-1107.
3. Chan LS: Human skin basement membrane in health and in autoimmune diseases. *Front Biosci*. 1997; 15: 343-352.
4. Uitto J, Pulkkinen L: Molecular complexity of the cutaneous basement membrane zone. *Mol Biol Rep*. 1996; 23: 35-46.
5. Abreu-Velez AM, Smith JG Jr, Howard MS: IgG/IgE bullous pemphigoid with CD45 lymphocytic reactivity to dermal blood vessels, nerves and eccrine sweat glands. *North Am J Med Sci*. 2010; 2: 538-541.
6. Abreu Velez AM, Girard JG, Howard MS: IgG bullous pemphigoid with antibodies to IgD, dermal blood vessels, eccrine glands and the endomysium of monkey esophagus. Ms in press. *N Dermatol Online*. *N Dermatol Online* 2011; 2: 48-51.
7. Howard MS, Yepes MM, Maldonado-Estrada JG, Villa-Robles E, Jaramillo A, Botero JH, et al: Broad histopathologic patterns of non-glabrous skin and glabrous skin from patients with a new variant of endemic pemphigus foliaceus-part 1. *J Cutan Pathol*. 2010; 37: 222-223.
8. Abreu Velez AM, Howard MS, Hashimoto T: Palm tissue displaying a polyclonal autoimmune response in patients affected by a new variant of endemic pemphigus foliaceus in Colombia, South America. *Eur J Dermatol*. 2010; 20:74-8.
9. Abreu-Velez AM, Howard MS, Hashimoto T, Grossniklaus HE: Human eyelid meibomian glands and tarsal muscle are recognized by autoantibodies from patients affected by a new variant of endemic pemphigus foliaceus in El-Bagre, Colombia, South America. *J Am Acad Dermatol*. 2010; 63: 437-447.
10. Abreu Velez AM, Howard MS, Hashimoto K, Hashimoto T: Autoantibodies to sweat glands detected by different methods in serum and in tissue from patients affected by a new variant of endemic pemphigus foliaceus. *Arch Dermatol Res*. 2009; 301: 711-718.
11. Abreu-Velez AM, Howard MS, Yi H, Gao W, Hashimoto T, Grossniklaus HE: Neural system antigens are recognized by autoantibodies from patients affected by a new variant of endemic pemphigus foliaceus in Colombia. *J Clin Immunol*. 2011;3:356-368.
12. Abreu Velez AM, Avila IC, Segovia J, Yepes MM, Bollag WB: Rare clinical form in two patients affected by a new variant of endemic pemphigus in northern Colombia. *Skinmed*. 2004; 3: 317-321.
13. Jordon RE, Sams WM Jr, Beutner EH: Complement immunofluorescent staining in bullous pemphigoid. *J Lab Clin Med*. 1969; 74: 548-556.
14. Yang YW, Chen YH, Xirasagar S, Lin HC: Increased Risk of Stroke in Patients With Bullous Pemphigoid: A Population-Based Follow-Up Study. *Stroke*. 2011; 42: 319-323.
15. Langan SM, Groves RW, West J: The relationship between neurological disease and bullous pemphigoid: a population-based case-control study. *J Invest Dermatol*. 2011; 3: 631-636.
16. Cordel N, Chosidow O, Hellot MF, Delaporte E, Lok C, Vaillant L, et al: Neurological disorders in patients with bullous pemphigoid. *Dermatology*. 2007; 215: 187-191.
17. Foureur N, Descamps V, Lebrun-Vignes B, Picard-Dahan C, Grossin M, Belaich S, et al: Bullous pemphigoid in a leg affected with hemiparesia: a possible relation of neurological diseases with bullous pemphigoid? *Eur J Dermatol*. 2001; 11: 230-233.
18. Tseng KW, Peng ML, Wen YC, Liu KJ, Chien CL: Neuronal degeneration in autonomic nervous system of dystonia musculorum mice. *J Biomed Sci*. 2011. 28: 9.
19. Magro CM, Dyrsen ME: The use of C3d and C4d immunohistochemistry on formalin-fixed tissue as a diagnostic adjunct in the assessment of inflammatory skin disease. *J Am Acad Dermatol*. 2008; 59: 822-833.
20. Pfaltz K, Mertz K, Rose C, Scheidegger P, Pfaltz M, Kempf W: C3d immunohistochemistry on formalin-fixed tissue is a valuable tool in the diagnosis of bullous pemphigoid of the skin. *J Cutan Pathol*. 2010; 37: 654-658.

21. Ameglio F, D'Auria L, Cordiali-Fei P, Mussi A, Valenzano L, D'Agosto G, et al: Bullous pemphigoid and pemphigus vulgaris: correlated behaviour of serum VEGF, sE-selectin and TNF-alpha levels. *J Biol Regul Homeost Agents*. 1997; 11: 148-153.

22. D'Auria L, Cordiali Fei P, Pietravallo M, Ferraro C, Mastroianni A, Bonifati C, Giacalone B, et al: The serum levels of sE-selectin are increased in patients with bullous pemphigoid or pemphigus vulgaris. Correlation with the number of skin lesions and recovery after corticosteroid therapy. *Br J Dermatol*. 1997; 137: 59-64.

23. Zebrowska A, Sysa-Jedrzejowska A, Wagrowska-Danilewicz M, Joss-Wichman E, Erkiert-Polgaj A, Waszczykowska E: Expression of selected integrins and selectins in bullous pemphigoid. *Mediators Inflamm*. 2007; 31051.

Funding source: Georgia Dermatopathology Associates, Atlanta, Georgia, USA