

## A CASE OF *BULLOUS PEMPHIGOID* WITH IMMUNOREACTIVITY TO BLOOD VESSELS AND SWEAT GLANDS

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### Abstract

**Introduction:** Bullous pemphigoid (BP) is one of the most prevalent autoimmune blistering diseases, and believed to be mediated by autoantibodies and complement. The disorder is categorized by the development of urticarial plaques surmounted by subepidermal blisters, and the deposition of immunoglobulins and complement at the basement membrane zone (BMZ) of the skin.

**Case Report:** A 70-year-old male Caucasian patient was evaluated for a four day history of multiple itchy, erythematous blisters on his abdomen. Biopsies for hematoxylin and eosin (H&E) examination, immunohistochemistry (IHC) and direct immunofluorescence (DIF) analyses were performed.

**Results:** The H&E biopsy demonstrated a subepidermal blister, with partial re-epithelialization of the blister floor. Within the blister lumen numerous neutrophils and eosinophils, and occasional lymphocytes were observed. Within the dermis, dilated superficial blood vessels with a mild, perivascular infiltrate of lymphocytes, histiocytes and eosinophils were seen; mild perivascular leukocytoclastic debris was also noted. A periodic acid Schiff (PAS) special stain demonstrated positive staining along the BMZ, and around selected dermal blood vessels and sweat glands. DIF revealed linear deposits of IgG, Complement/C3 and fibrinogen at the BMZ, and around selected dermal blood vessels and sweat glands. By IHC, positive staining for CD8 and CD45, and occasional CD4 positivity was seen on dermal lymphocytes. These lymphocytes were present surrounding selected dermal blood vessels and eccrine sweat glands.

**Conclusions:** The patient displayed immunoreactivity to the BMZ, and also to dermal blood vessels and eccrine glands in his immune response. Similar immune responses would be of interest in immunologic studies of BP patients.

**Key words:** bullous pemphigoid; blood vessels; sweat glands; autoantibodies

**Abbreviations and acronyms:** Bullous pemphigoid (BP), immunohistochemistry (IHC), direct and indirect immunofluorescence (DIF and IIF), hematoxylin and eosin (H&E), basement membrane zone (BMZ), intercellular staining between epidermal keratinocytes (ICS).

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### Introduction

Patients with bullous pemphigoid (BP) have autoantibodies binding to specific antigens in the basement membrane zone (BMZ), causing detachment of the entire epidermis from the dermis [1]. The autoantibodies directed against the BMZ can be visualized by both direct and indirect immunofluorescence (DIF, IIF) [2-4]. Eosinophils, neutrophils, and mast cells have all been implicated in the pathogenesis of BP [5-7]. It is also believed that the autoantibodies in turn activate complement and other inflammatory mediators, causing mast cell degranulation and migration of eosinophils, neutrophils

and antigen presenting cells to the BMZ. These events then lead to splitting at the dermal/epidermal junctional zone [1]. Specifically, BMZ splitting is thought to occur secondary to secretion of inflammatory cytokines and proteases.

Clinically, bullous pemphigoid (BP) is a rare skin disorder that causes tense, fluid-filled blisters on the lower abdomen, upper thighs, armpits and abdomen. BP is most common in people older than age 60 [1,2]. Treatment frequently includes corticosteroids such as prednisone, and other drugs that suppress the immune system. BP can be life-threatening, especially for older people who are already in poor health.

## Case Report

A 70 year old male patient was referred with a four day eruption of multiple, severely pruritic, tense bullae with erythematous bases concentrated on the abdomen (Fig. 1a). Skin biopsies for hematoxylin and eosin (H&E), direct and indirect immunofluorescence (DIF and IIF) and immunohistochemistry (IHC) review were performed. In addition, IIF with 0.1 M sodium chloride salt split skin was requested.

## Methods

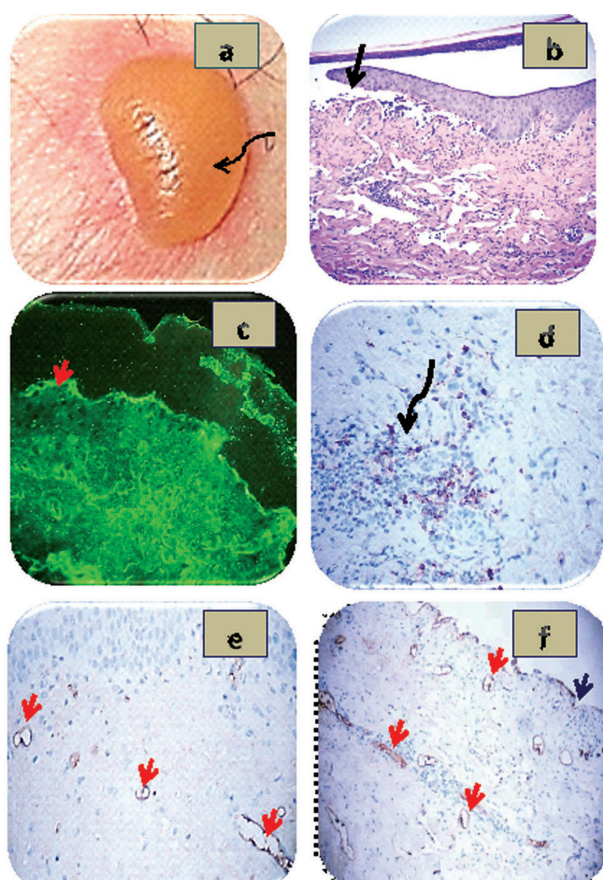
### DIF

In brief, our DIF was performed utilizing skin cryosections, incubated with multiple fluorescein isothiocyanate (FITC)-conjugated secondary antibodies as previously described [8,9]. The secondary antibodies were of rabbit origin, and included a) anti-human IgG, b) anti-human IgA, c) anti-human IgM, d) anti-human fibrinogen, and e) anti-human albumin (all with at 1:20 to 1:40, anti-human C3C and C4 FITCI conjugates obtained from Dako (Carpinteria, California, USA)). We also utilized FITC conjugated secondary antibodies of goat origin, including a) anti-human IgE (Vector Laboratories, Bridgeport, New Jersey, USA) and b) anti-human complement/C1q and IgD FITCI conjugated (Southern Biotech, Birmingham, Alabama, USA). The DIF slides were counterstained with 4',6-diamidino-2-phenylindole (Dapi) (Pierce, Rockford, Illinois, USA) washed, coverslipped, and dried overnight at 40C. The NACI split skin as performed as previously described [8,9]. IHC staining was performed using anti-human antibodies to IgG, IgM, IgE, Complement/C3c, fibrinogen, CD4, CD8, CD45, kappa light chains, lambda light chains and myeloid/histiocyte antigen (Clone MAC 387). Our IHC antibodies were all obtained

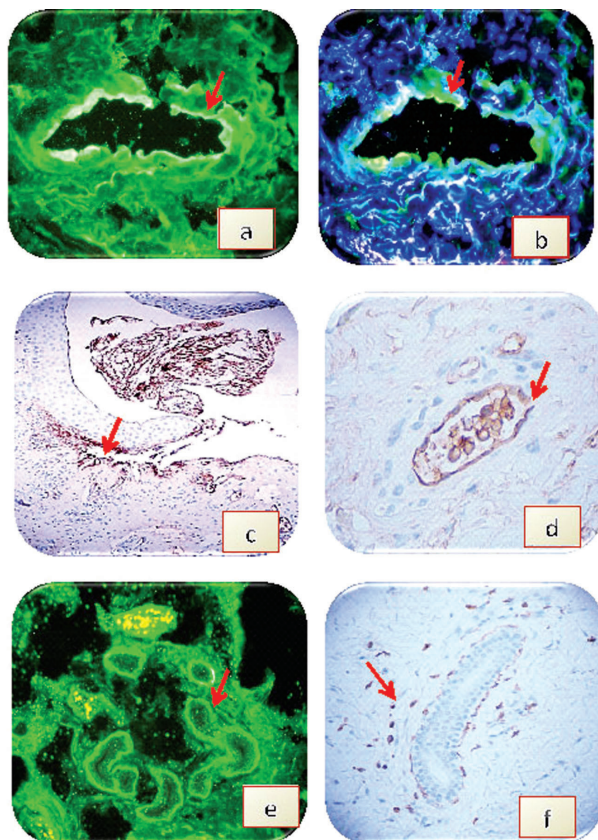
from Dako. Our IHC staining was performed utilizing a Dako automatized dual endogenous flex system, and following Dako technical instructions as previously described [8,9].

## Result

Examination of the H&E tissue sections demonstrated a tense subepidermal blister. The edge of the blister demonstrated degranulating eosinophils, close to the epidermal BMZ. Within the dermis, a mild, superficial, perivascular infiltrate of lymphocytes, histiocytes, eosinophils was identified. The vessels in the upper dermis were dilated. A PAS special stain was reviewed; the positive control stained appropriately. The PAS special stain revealed no fungal organisms, and focally increased staining around dermal blood vessels. DIF displayed the following results: IgG (+++, linear BMZ); IgA (+, focal linear BMZ); IgM (+, Focal dermal perivascular cells); IgD(-); IgE (-); complement/C1q (+ focal dermal perivascular cells); complement/C3 (+++, linear BMZ); complement/C4 (-); albumin (+, focal epidermal cell junctions and focal linear BMZ) and Fibrinogen(+, focal BMZ and diffuse deep dermal). IIF showed similar results as DIF, and IgG titers of 1:280. Salt split skin/IIF studies revealed that the primary autoreactivity was on the blister roof; however, focal staining was also noted on the blister floor. Finally, by IHC the dermal blood vessels stained positively for IgG (++), IgM (+++), IgE (+), fibrinogen (+++) and complement/C3(++). Lymphocytes surrounding these blood vessels stained positively for CD4(+), CD8(++) and CD45(+++). The dermal eccrine sweat glands and ducts stained positive for IgM(+++) and lambda light chains (++). In Figures 1 and 2, we highlight our most significant H&E, DIF and IHC results.



**Figure 1.** a. A representative clinical blister(black arrow). b. A representative H&E section, demonstrating a subepidermal blister (black arrow) (100X). c. DIF positive stain with FITC conjugated fibrinogen, present in a linear pattern at the BMZ (green staining; black arrow). Also note the focal positive staining around upper dermal blood vessels and within the epidermis. d. Positive IHC staining for IgE around an upper dermal vessels (red/purple staining; black arrow). e. Positive IHC staining for IgG around small upper dermal blood vessels, (brown staining; red arrows). f. Positive IHC staining for IgM around small upper dermal blood vessels (brown staining; red arrows). The blue arrow highlights additional linear staining on the floor of the BP blister.



**Figure 2.** a. Positive DIF staining with FITC conjugated fibrinogen against a blood vessel in the dermis (green/white staining; red arrow)(400X). b. Same as a, but adding a Dapi counterstain to highlight blood vessel endothelial cell nuclei (blue staining) (400X). c. IHC positive staining for IgM inside a BP blister lumen, as well as along the BMZ(brown staining; red arrow) (100x). d. IHC positive staining for IgG on a dermal blood vessel interior surface (brown staining; red arrow). e. DIF, showing positive staining with FITC conjugated Complement/C3c on a dermal eccrine gland coil (green staining; red arrow). f. IHC positive staining with myeloid/histiocyte antigen antibody around an eccrine sweat duct (brown staining; red arrow).

## Discussion

Bullous pemphigoid is a subepidermal bullous dermatosis resulting in a pathologic disruption between basaloid layer of the epidermis and the dermis, and thus causing formation of tense clinical blisters [1]. Many previous studies have documented an increase in blood vessel permeability in BP [5-7]. It is known that the human cutaneous BMZ contains multiple components, including BPAGI (230kD) and BPAGII (180 kDa; Collagen Type XVII) proteins; Type I, IV and VII collagens, alpha6 and beta4 integrins, laminins 1, 5 and 6, entactin/nidogen, heparan sulfate proteoglycans and microfibrils. The dermal blood vessels also contain diverse molecules, including laminin 1, Type IV collagen, and heparan sulfate proteoglycans. Thus, it is possible to hypothesize that any of these antigens in the skin and dermal blood vessels could be immune targets in BP. We previously reported a different case of BP, having autoantibodies to dermal blood vessels and sweat glands [10]. It has also been reported that Collagen XVII is expressed in podocytes of the renal glomerular barrier [11]. We have also previously observed strong activity of several proteases and protease inhibitors in the dermal blood vessels in BP, as well as in other autoimmune blistering diseases [12].

We were able to demonstrate a direct correlation of our DIF and IIF/salt split skin positive findings with positive PAS staining in dermal blood vessels. Notably, vascular dilatation and perivascular dermal infiltrates have been previously documented in BP. Recently, we reported that soluble E-selectin (sE-selectin; an isoform of cell membrane E-selectin, an adhesion molecule synthesized only by endothelial cells), is significantly increased in sera of patients with BP and pemphigus vulgaris. We also reported that collagen XVII (a transmembrane molecule known

to be required for epithelial adhesion) is expressed in podocytes of normal human and mouse renal tissue, as well as in endothelial cells of the glomerular filtration barrier. Immunoelectron microscopy has revealed that the collagen XVII is specifically localized in the foot processes of the podocytes, and within the glomerular basement membrane [12].

Multiple authors have previously reported augmentation of chemokines, cytokines and ICAM/CD54 in BP; in addition, increased expression of vascular permeability factors including integrins and selectins in BP has been documented [13-23].

We conclude that our findings of 1) abundant IHC CD45 positive lymphocytes around the dermal blood vessels and eccrine glands and 2) positive autoantibody staining observed by DIF and IIF in these areas warrant additional investigation in BP cases.

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