ABSTRACT

The immunobullous disorders are a group of autoimmune diseases in which components of the epidermis and basement membrane zone are targeted, resulting in the formation of cutaneous and mucosal blisters. Based on the level of blistering, the autoimmune blistering diseases may be subdivided into intraepidermal and subepidermal. An exhaustive list of immunobullous disorders is beyond the scope of this review, but those involving oral mucosa are taken into consideration.

One major group namely the subepidermal immunobullous diseases which includes bullous pemphigoid (BP), mucosal pemphigoid [cicatricial pemphigoid (CP) or (MMP)], epidermolysis bullosa acquisita (EBA) linear IgA bullous disease (LABD) are discussed in this section. The diagnosis of these diseases requires clinicopathological correlation; immunofluorescence methods provide a useful adjunct to light microscopy. These methods entail the use of fluorescein-linked antibodies to immunoglobulins, complement components, or other proteins either in the skin biopsy or sera. In continuation with part I, the immunofluorescence patterns in the above listed immunobullous disorders are reviewed in detail with a summary of pathogenesis and characteristic histopathological findings.

Keywords: Subepidermal immunobullous diseases, Immunofluorescent patterns, Oral mucosa.

INTRODUCTION

The stratified squamous epithelium of the human epidermis forms a continuous barrier against the external environment. The pathophysiology of blistering diseases illustrates how impairments in epithelial adhesion lead to disorders characterized by substantial morbidity and/or mortality. Blistering diseases can be inherited or acquired; most examples of the latter are autoimmune in nature and are characterized by autoantibodies that target adhesion junctions promoting either cell-cell or cell-matrix adhesion in skin.1 Patients with bullous pemphigoid (BP) and other autoimmune subepidermal blistering diseases have autoantibodies that target autoantigens in epidermal basement membrane (BM)2,3 (Fig. 1 and Table 1).

Fig. 1: Ultrastructural subregions of the epidermal basement membrane and representative autoantigens of corresponding immunobullous diseases. AECP: Antiepiligrin cicatricial pemphigoid; CP: Cicatricial pemphigoid; EB: Epidermolysis bullosa; IB: Immunobullous; LAD: Linear IgA dermatosis; OCP: Ocular cicatricial pemphigoid
Circulating autoantibodies can be demonstrated in the patient’s skin biopsies by direct immunofluorescence techniques (DIF) and in the patient’s serum by indirect immunofluorescence technique (IIF), the techniques and methodologies have been reviewed exhaustively in the part I of this series. The relative simplicity, accuracy and the combination of the specificity of immunology with the localization of histopathology has made immunofluorescence an indispensable technique in the diagnosis of immunobullous diseases.

In this part, the main theme is focused on pathology and immunofluorescent patterns in subepidermal immunobullous disorders of pemphigoid group involving oral cavity. The pemphigoid group of diseases has been classified into two main clinical subgroups: Bullous pemphigoid, which involves skin but 30% of the time involves oral mucous membranes as well; and cicatricial pemphigoid (mucous membrane pemphigoid), which involves mucous membranes (usually oral or conjunctival) and skin to lesser extent (20%). Today, this group of diseases is categorized into two main clinical groups: Cutaneous and mucosal pemphigoid. The mucosal pemphigoids are subclassified into three subgroups as follows: Cicatricial pemphigoid, oral mucous membrane pemphigoid and ocular pemphigoid.4

### BULLOUS PEMPHIGOID (CUTANEOUS PEMPHIGOID)

Bullous pemphigoid (BP) is the most common of all autoimmune blistering conditions. It is primarily a skin disease, forming oral lesions in only 8 to 39% of cases.4,5

### Pathogenesis

Blister formation is found to occur within the lamina lucida of the basement membrane, causing a loss of anchoring filaments and hemidesmosomes. There are IgG autoantibodies specific for the hemidesmosomal BP antigen BP230 (BPAg1) and BP180 (BPAg2).6,7 The binding of the antibodies at the basement membrane activates complement and inflammatory mediators. Although BPAg2 has been identified as the major antigen involved with BP disease development, autoantibodies against α6 integrin and laminin-5 were identified in patients affected by BP. IL-5, an interleukin with eosinophil chemoattractant and activation properties has been found in the skin of patients with BP.4,5,8

### Histopathology

The perilesional margin of a bulla shows separation of the epithelium from the connective tissue at the basement zone, resulting in subepithelial separation.9 The BM remains attached to the connective tissue rather than to the overlying separated epithelium. Modest numbers of both acute and chronic inflammatory cells are typically seen in the lesional area, and the presence of eosinophils within the bulla itself is characteristic.5,10 (Fig. 2).

### Immunofluorescent Patterns and Its Significance

**DIF:** Testing of perilesional and uninvolved skin shows a thick linear band of C3 deposition along the basement membrane zone (BMZ) at the dermoeipidermal junction in virtually 100% of cases (Fig. 3) and IgG in 65 to 95%.5,11-14 (Fig. 4).

Salt-splitting direct techniques enhance the sensitivity of immunoreactant detection and help to differentiate between the various immunobullous disorders with immunoreactants deposited at the basement membrane zone.13 IgG will localize to the roof of the split in the

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Table 1: Immunofluorescence findings in immunobullous disorders46,47
**Majority of patients, to both roof and floor in 10% but occasionally to the floor alone.**

C3 will always bind to both roof and floor.18

Circulating IgG antibodies may be found in 95% of cases by using salt-split skin as a substrate.19 The antibodies are predominantly IgG1 and IgG4 subclasses.20 The use of salt-split skin substrate will usually reveal BP antisera binding to the epidermal side alone or to both epidermal and dermal aspects but occasionally antisera will bind only to the dermal aspect. In such cases, toad skin substrate may be utilized to confirm the diagnosis as it contains the bullous pemphigoid antigens.21 Thus, selection of substrate plays an important role in the detection of circulating antibodies.22

**IIF**: Studies reveal circulating antibasement membrane zone IgG antibodies in 70 to 80% of cases. Similarly deposition of IgG and IgM are observed in about 25% of cases. Correlation exists between the antibody titer and the clinical severity of the disease.23 The IgG is located within the lamina lucida.1,4

**MUCOSAL PEMPHIGOIDS**

**Cicatricial Pemphigoid or Mucous Membrane Pemphigoid (MMP)**

Pemphigoid came to include cicatricial pemphigoid now renamed as mucous membrane pemphigoid MMP lesions in the oral cavity are present in 85% of patients. The oral mucosa is often the initial site of MMP lesions. Desquamative gingivitis is the main oral feature of MMP.24

**Variants of MMP with Oral Lesions**

At least six variants or subsets of MMP with different antigenic specificity of autoantibodies and patterns of immunopathology are now recognized, but new immune-mediated, subepithelial, blistering diseases with oral lesions that simulate have also been described.24

The main oral variants are as follows

1. **Oral pemphigoid or OMMP (Oral lesions only)**: The target antigen for oral pemphigoid is still unclear; though antibodies against a 168-kDa oral mucosal protein have been seen in a few patients.25 It has a low incidence of findings on indirect immunofluorescence.26

2. **Pemphigoid with more widespread clinical features (oral and extraoral lesions)**: Anti-epiligrin pemphigoid (AECP): Blisters in mucous membrane and skin are rare and characterized by serologic reactivity only to the dermal side of salt-split skin. The targeted antigens are subunits of laminin 5 (epiligrin).27

**Pathogenesis**

The pathogenesis of MMP probably includes an autoantibody-induced complement-mediated sequestration of leukocytes (mainly neutrophils) with a resultant release
of cytokines and leukocyte enzymes, and the detachment of the basal cells from the basement membrane zone, and possibly some complement-mediated lysis of cells.24

The known autoantigens include (a) epiligrin or laminin 5, (b) BPAg2 and BPAl. All of these antigens occur within the lamina lucida, with epiligrin present in the lower lamina lucida. The autoantibodies involved in MMP are directed against these antigens, either in the basement membrane or hemidesmosomes.7,28

Histopathology
Histologically, MMP is characterized by junctional separation at the level of the basement membrane that gives rise to a sub-basilar split with a chronic inflammatory infiltrate in the lamina propria that contains eosinophils, lymphocytes and neutrophils.4,24

Immunofluorescent Patterns and Its Significance

DIF: Perilesional skin and mucosae have linear deposition of IgG and C3 along the BMZ in 80% of cases in a homogeneous manner. IgA and IgM are deposited less often. Deposition of immunoreactants along the BMZ of mucosal mucous glands appears to be a specific finding in cicatricial pemphigoid.24,29,30

IIF: Positive circulating antibodies are detected by IF and immunoblot assays in 5% patients. IIF testing of serum yields variable results depending on the substrate used (monkey esophagus, guinea pig esophagus, normal human skin, salt-split skin). Circulating antibodies may be readily demonstrated when salt-split human skin is used as substrate, in which IgG may be localized only to the roof.31

Indirect immunofluorescence using salt-split mucosa provides a more sensitive assay, can show antibasement membrane zone antibodies, and distinguishes between antigens on the epithelial side of the split (4 integrin and BPAg2) and those on the lamina propria side (laminin 5). Immunoblot assays are more specific than IF.24

Circulating IgG and IgA antibodies are usually of low titer and are detected in 20 to 30% with standard IIF methods but this may be increased to 80% by the use of salt-split skin substrate. IgM antibodies may also be found. The binding of antibodies is most commonly to the epidermal aspect of salt-split skin but may be to both sides or to the dermal side alone.19 IgG antibodies are usually IgG and IgG4 while IgA antibodies are always of IgA1 subclass. Titers do not relate to disease extent nor activity.29,32

EPIDERMOLYSIS BULLOSA ACQUISITA (EBA)

It is an IgG-mediated autoantibody disease with oral lesions present in nearly 50% of the cases reported along with cutaneous lesions.5

Pathogenesis
It is an immunologically-mediated condition characterized by autoantibodies directed against type VII collagen, the principle component of the anchoring fibrils. As a result, their immunologic destruction results in the formation of bullous lesions of the skin and mucosa with minimal trauma.5

Histopathology
A cell-poor subepidermal split with variable dermal cellular infiltration is seen on biopsy.33

Immunofluorescent Patterns and Its Significance

DIF: IgG is deposited linearly along the BMZ of perilesional skin in all active cases. IgA, IgM and C3 are also often present. Salt-splitting DIF techniques reveal a dermal pattern of immunoreactant deposition in all cases.34,35

IIF: The detection and titer of circulating IgG anti-BMZ antibodies may be increased from 25 to 50% using standard IIF methods 34, 35 to and 50 to 85% with salt-split skin substrate.36,37 The antibodies in EBA have specificity for the globular carboxyl terminus of type VII collagen and are deposited beneath the lamina densa.38,39 Therefore, on salt-split skin studies, IgG is on the floor and not on the roof of the split.40

Linear IgA Disease

Linear IgA disease is a rare autoimmune disease involving skin and oral mucosa. It is not considered a part of the pemphigoid group but is a similar disease in which autoantibodies attack basement membrane proteins.4

Histopathology
The distinguishing feature of linear IgA disease is the presence of IgA in a homogeneous, linear pattern at the basement membrane zone in lesional and perilesional tissue. The vesicles are subepithelial and occur within the lamina lucida or below the basal lamina.4

The histology is in many cases identical to that found in dermatitis herpetiformis but may also resemble bullous pemphigoid.33

Immunofluorescent Patterns and Its Significance

DIF: Reveals linear IgA along the basement membrane zone in the perilesional skin in 100% of cases. It has been suggested that if IgA deposits are more intense than the
IgG deposits, and C3 deposition is strong, then linear IgA dermatosis is the best diagnosis.14,41,42

Epidermal, dermal and combined patterns of IgA deposition may be seen with salt-splitting of the biopsy.43 Children more commonly have positive indirect immunofluorescence than adults with figures of 72% and 20% respectively obtained in one study using standard techniques.44 Salt-split skin substrate will increase the detection rate and titer of antibodies with binding to either the roof or floor, the former being more common.45,46

CONCLUSION

Immunofluorescence combines the specificity of serology and localization of histology. Thus, it helps in visualization of antigen-antibody reaction in situ. It is an invaluable tool in the diagnosis of immunobullous lesions.

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