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REVIEW ARTICLE

Immunofluorescence in Oral Pathology—Part III: Pathology and Immunofluorescent Patterns in Intraepithelial Immunobullous Disorders

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ABSTRACT

Dermatologic disorders frequently parallel with oral involvement which dental practitioners should be familiar with. In continuation with part II, the immunofluorescence patterns in the intraepidermal pemphigus group and its variants: Pemphigus vulgaris (PV) and paraneoplastic pemphigus (PNP) are dealt in this section. Also, a brief note is added on other mucocutaneous disorders showing similar immunofluorescence patterns. The above listed immunobullous disorders are reviewed in detail with a summary of pathogenesis and characteristic histopathological findings. This review is to facilitate the clinicopathologist in the early diagnosis and subsequent treatment of these debilitating conditions.

Keywords: Intraepidermal immunobullous diseases, Immunofluorescence patterns, Oral mucosa.

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INTRODUCTION

Immunobullous diseases constitute an important group of dermatological disorders caused by autoantibodies directed against antigens in the intercellular substance or dermoepidermal/epithelial junction resulting in the formation of cutaneous and mucosal blisters.[1] Immunobullous diseases with oral mucosal involvement can be divided into intraepithelial/intraepidermal and subepithelial/subepidermal. The intraepithelial diseases include the pemphigus group and its variants: Pemphigus vulgaris (PV), pemphigus foliaceus (PF), etc. The subepithelial immunobullous group of lesions was discussed in detail in part II of this series. The intraepidermal immunobullous disorders and other mucocutaneous disorders sharing similar immunofluorescence patterns namely lichen planus (LP), systemic lupus erythematosus (SLE), discoid lupus erythematosus (DLE) and erythema multiforme (EM) are dealt in this section.

PEMPHIGUS GROUP OF LESIONS

It includes a group of chronic mucocutaneous, autoimmune blistering diseases that develop in cycles characterized by intraepithelial blisters histologically, and immunologically, circulating IgG directed against the cell surface of keratinocytes. Pemphigus can be classified into 6 types: pemphigus vulgaris (PV), pemphigus vegetans, pemphigus erythematosus, pemphigus foliaceus (PF), paraneoplastic pemphigus (PNP), and immunoglobulin IgA pemphigus.[2,3] Pemphigus vulgaris is the main variant followed by PNP that usually affects the mouth and precedes skin lesions in most cases.[3]

PEMPHIGUS VULGARIS

Pemphigus vulgaris is an autoimmune mucocutaneous disorder mediated by circulating autoantibodies directed against keratinocyte cell surfaces. Oral lesions are seen in 90% of patients, usually at an early stage and can precede cutaneous lesions in weeks, months or more than 1 year.

Histopathology

The histopathological features of PV reflect the action of the circulating antibody on the cell surface of the prickle cells and consequent destruction of the desmosomes termed as acantholysis. This results in the formation of clefts and then blisters or bullae in a predominantly suprabasal location[4-8] (Fig. 1). The acantholytic cells called Tzanck cells are found in the vesicular space (Fig. 2). Sometimes the entire superficial layers of the epithelium are stripped away, leaving only the basal cells forming a 'row of tomb stones' as the basal cells are firmly attached to the basement membrane by hemidesmosomes[5,6,8] (Fig. 3).

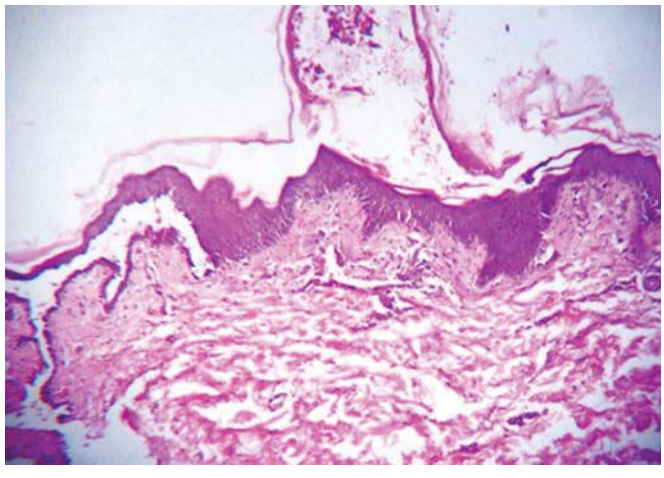

Fig. 1: Pemphigus vulgaris: H and E stained section at 4× showing a suprabasal blister

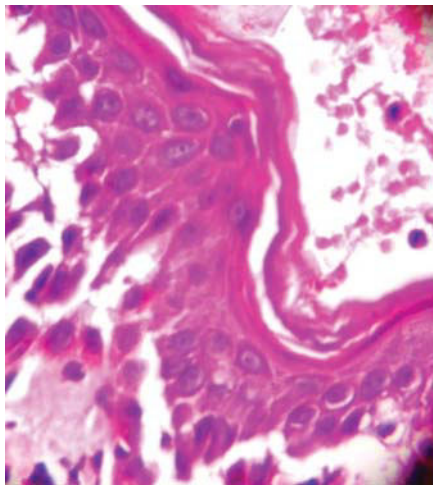

Fig. 2: Pemphigus vulgaris: H and E stained section at 40× showing acantholytic cells in the suprabasal blister

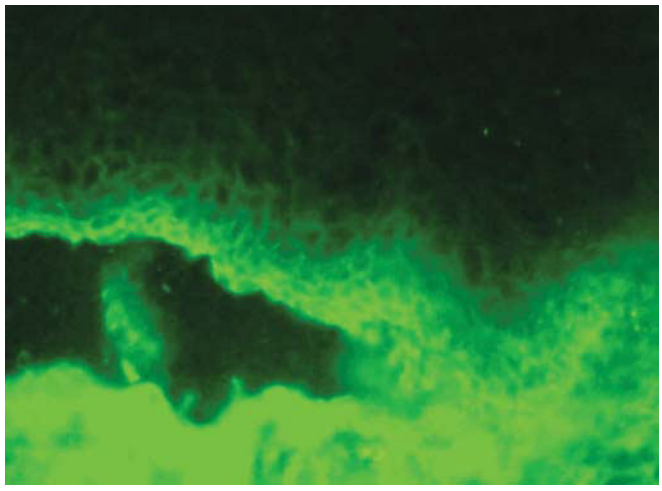

Fig. 4: Pemphigus vulgaris: Direct immunofluorescence showing the suprabasal cleavage. Lace-like deposition of IgG is seen in the squamous intercellular spaces in the lower epidermis *(Courtesy*: Dr HC Mahadeva and Dr Arundati)

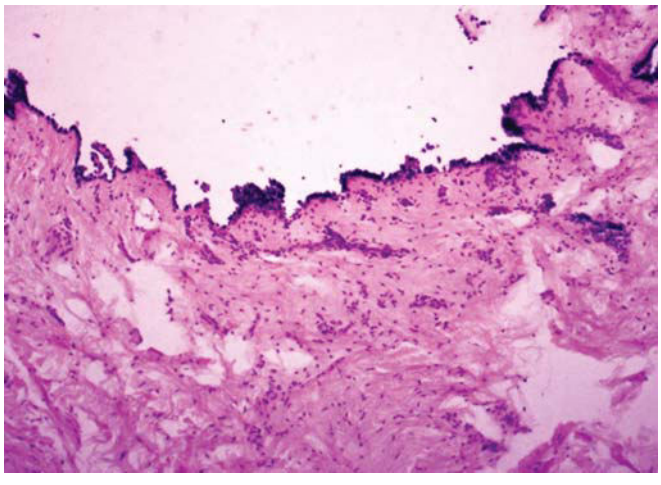

Fig. 3: Pemphigus vulgaris: H and E stained section at 10x showing dermal papillae (villi) lined by a single layer of preserved basal keratinocytes presenting a 'row of tomestones' appearance

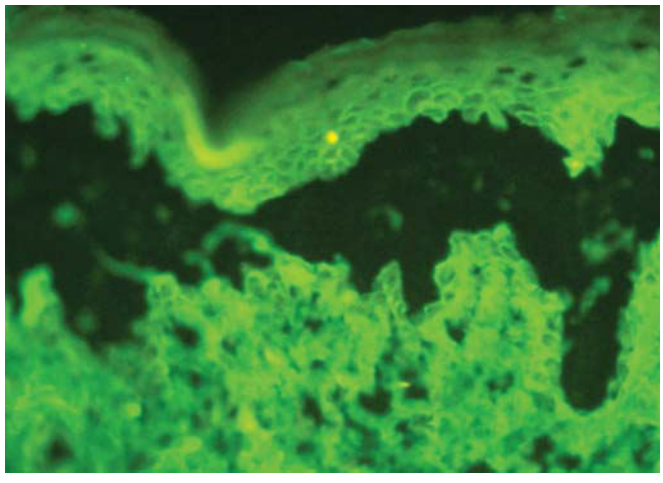

Fig. 5: Pemphigus vulgaris: Direct immunofluorescence showing the suprabasal cleavage. Lace-like deposition of C3 is seen in the squamous intercellular spaces in the lower epidermis (*Courtesy*: Dr HC Mahadeva and Dr Arundati)

A feature that is often helpful diagnostically is the villous projections that may develop at the base of the bulla. When the surface epithelium ultimately separates, the mucosa will appear to have a surface of papillary projections lined by basal cells with some acantholytic cells. Once the bulla ruptures, the underlying connective tissue becomes highly inflammed. The eosinophilic infiltrate often seen in skin occurs infrequently in oral mucosa.[9]

Pathogenesis

The etiology and pathogenesis of PV are not completely clear. Compelling evidence has accumulated that IgG serum autoantibodies are directed against a cadherin-type cell adhesion molecule in stratified squamous epithelia, called desmoglein 3 and 1.[10] In PV IgG antibodies are directed particularly to desmoglein 3, and since oral epithelium expresses largely desmoglein 3, oral lesions appear at an early stage. The presence of antibodies against desmoglein 1 (found in over 50% of cases) may result in cutaneous involvement. Damage to the intercellular area between keratinocytes leads to apoptosis resulting in separation of the keratinocytes, acantholysis (cleavage) and then blistering.[10] This occurs mostly at the level just superficial to basal cells.[5] Antibody binding may activate complement with release of inflammatory mediators and recruitment of activated T cells.[11]

Immunofluorescent Patterns and its Significance

Direct immunofluorescence (DIF) testing is a very reliable and sensitive diagnostic test for pemphigus vulgaris. IgG or IgM and C3 expression are present in the squamous intercellular/cell surface areas in up to 95% of cases, including early cases and those with very few lesions.

Intercellular expression of IgG or IgM and C3 are present in up to 100% of cases with active disease.[12] IgG deposits are greater than IgM, IgA and C3. Fluorescence of greatest intensity is seen in the parabasal region. Chicken wire/fishnet pattern of deposition is noted around spinous cells of the epithelium in intercellular spaces.[1,5,11] Complement component C3 may be seen in a pattern similar to that of IgG, but with lower frequency and intensity. However, the diagnosis of pemphigus should not be made when only C3 is deposited[1] (Figs 4 and 5).

Direct immunofluorescence (DIF) may be used to assess disease activity during therapy-induced remission. Negative DIF findings may be a good prognostic indicator when the patient is in remission.[13]

Indirect immunofluorescence (IIF) studies during active disease reveal an IgG antibody to the intercellular cement substance of stratified cornified epithelium in almost 90% of cases.[14] The calcium enhancement technique will enhance detection of these antibodies whose titer reflects disease activity.[15,16] A two-fold or greater increase in the titer suggests impending relapse.[17] The absolute titer may be a poorer predictor of clinical status than the trend over some time although very high or absent levels usually correlate with active or inactive disease respectively.[18] Antibodies are predominantly IgG4 subclass.[19]

PARANEOPLASTIC PEMPHIGUS

Paraneoplastic pemphigus (PNP) is a specific paraneoplastic disorder most commonly associated with lymphomas.[20] Oral mucosal involvement is the earliest feature of PNP.

Histopathology: Histology shows suprabasal acantholytic separation, satellite keratinocyte necrosis, basal cell vacuolation, spongiosis and a lymphoid infiltrate at the epithelial-connective tissue interface.[9,21,22]

Pathogenesis

Patients with PNP develop IgG autoantibodies against multiple antigens. Mainly members of the plakin family, as well as desmogleins, are targeted by IgG autoantibodies in PNP. Antidesmoglein antibodies play a role in inducing the loss of cellular adhesion of keratinocytes and blister formation. The intracellular location of plakin proteins makes them unlikely initial targets. The damage to cell membranes induced by antidesmoglein IgG provides access into the cell for antiplakin autoantibodies; the latter then bind to their target antigens, inhibit their functions, and perhaps precipitate some unique features of PNP, such as dyskeratosis.[23] In addition to the humoral autoimmunity, autoreactive cellular responses mediated by CD8+ cytotoxic T-lymphocytes, natural killer cells, and monocytes/macrophages appear to be important in the pathogenesis of paraneoplastic autoimmune multiorgan syndrome.[23]

Immunofluorescent Patterns and its Significance

DIF studies may show weakly positive deposition of immunoreactants IgG and C3 almost invariably in the intercellular substance in perilesional skin and mucosa.[20-22] C3, IgG and IgM may also be deposited along the basement membrane zone.[20,22] However, false-negative DIF is more commonly noticed in PNP than in other forms of pemphigus.[1,5,9,11]

Indirect immunofluorescence testing using rat bladder transitional epithelium is highly specific for this disease.[5,9,24,25] Circulating IgG anti-intercellular substance antibodies are almost always present in high titer.[20]

In pemphigus, titers of circulating intercellular antibodies can be correlated with disease activity; a two-fold rise in titer may indicate an impending relapse. Of the four classes of IgG, IgG1 is the best indicator of disease.[26]

Other dermatological conditions sharing similar immunofluorescence patterns like lichen planus (LP) systemic lupus erythematosus (SLE), discoid lupus erythematosus (DLE) and erythema multiforme (EM) are discussed briefly in this section.

LICHEN PLANUS

Immunofluorescent Patterns and its Significance

Direct IF: The shaggy deposits react with anti-fibrinogen to exhibit intensely positive fluorescnece in the basement membrane zone.[27] Occasionally there are granular deposits of IgM or linear deposits of C3.[28] The colloid apoptotic cells can also be demonstrated. They typically stain for IgM, but staining with IgG, IgA, C3 and fibrin may also be seen.[9]

Indirect IF: Negative and can be used to demonstrate lichen planus specific antigen (LPSA), which is expressed in the stratum granulosum and stratum spinosum. LPSA is specific for lichen planus and is found in 80% of patients with and without oral lesions.[26]

BULLOUS SYSTEMIC LUPUS ERYTHEMATOSUS

Immunofluorescent Patterns and its Significance

Direct IF: IgG and C3 are deposited at the epidermal basement membrane zone. The pattern is linear, but sometimes may be 'shaggy' or 'granular band-like'. A linear rather than granular pattern along the BMZ is associated with the presence of higher titer of circulating auto-

antibodies.[29,30] IgM and IgA are present in approximately 50 to 60% of cases respectively. In general, granular patterns (60%) represent deposition of circulating immune complexes *in situ* or *in situ* binding of antigen and antibody in compartmentalized zones. Similar deposition of IgG, IgM or complement in the normal skin is known as positive lupus band test.[5]

Bullous SLE is associated with a higher incidence of IgA deposition (76%) than other forms of SLE (17%) and this may also correlate with renal involvement.[31] C3 is usually deposited in lesional skin.[30]

DISCOID LUPUS ERYTHEMATOSUS

Immunofluorescent Patterns

Direct IF: It is similar to SLE; most cases will demonstrate a granular pattern of IgG, IgM, IgA, C3 and fibrinogen in the basement membrane zone of involved mucosa or skin.[9] But, Ig can never be demonstrated in the uninvolved skin and mucosa in DLE.[11]

ERYTHEMA MULTIFORME

Immunofluorescent Patterns

Direct IF: By DIF, the superficial vessels of the connective tissue are seen to contain IgM and C3 in their walls. At the dermal-epidermal junction, granular deposits of IgM, fibrinogen and C3 may be seen. Circulating immune complexes have also been found.[9]

Indirect immunofluorescence is negative for circulating antibodies.

CONCLUSION

The gist of the entire article regarding antibodies, target antigen, structural target and immunofluorescence pzatterns of pemphigus and its variants and other mucocutaneous disorders sharing similar immunofluorescent patterns has been summarized in Table 1.

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Table 1: Immunofluorescence findings in intraepithelial immunobullous disorders[26,32]

Epidermal bullous diseases	*Antibodies*					*Target antigen*	*Structural target*	*Immunofluorescence patterns*
	IgG	IgM	IgA	C3	Fibrin			
Pemphigus group Mucosal PV	+ve	–ve	–ve	+ve	–ve	Desmoglein 3 (130 Kda)	Desmosomes	Ig-Chicken wire/fishnet pattern
Mucocutaneous PV						Desmoglein 3 and 1		C3-Chicken wire/ fishnet pattern
Paraneoplastic Pemphigus: PNP						Desmoglein 3 and 1 Periplakin, Desmoplakin and envoplakin		IgG—membrane fluorescence of Tzanck cells
Other dermal conditions with oral involvement								
SLE (Ig from uninvolved skin)	+ve	+ve	–ve	+ve	–ve	—		Coarse granularity at BMZ
DLE (Ig from effected skill)	+ve	+ve	+ve	+ve	+ve			Coarse granularity at BMZ
Lichen planus	–ve	±ve	–ve	±ve	+ve	—		Fibrinogen: Shaggy/ fibrillar pattern C3: Fine granular deposition (not diagnostic) Rarely IgM, IgA, IgG, C3 +ve colloid bodies located in the epithelium and superficial connective tissue
Erythema multiforme	–ve	–ve	–ve	+ve	–ve IIF: –ve			Nondiagnostic IgM: Perivascular deep in connective tissue

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Photo courtesy: We are greatful to Dr KC Mahadeva, Professor and Head, Department of Pathology, MS Ramaiah Medical College and Dr Arundati, Tutor, Department of Pathology, MSRMC, Bangalore, for having helped us in procuring the photographs.

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