REVIEW

IMMUNOFLUORESCENCE OF THE IMMUNOBULLOUS DISORDERS. PART TWO : THE CLINICAL DISORDERS

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The immunofluorescence findings in the immunobullous disorders are reviewed together with a summary of clinical and histopathological findings.

Key Words : Dermatitis herpetiformis, Epidermolysis bullosa acquisita, Herpes gestationis, Immunofluorescence, Immunobullous disorders, Linear IgA disease, Pemphigoid, Pemphigus

Introduction

The immunobullous disorders are a group of autoimmune diseases in which components of the epidermis and basement membrane zone are the focus of attack, resulting in the formation of cutaneous and mucosal blisters. Early diagnosis and treatment of these severe and potentially life-threatening disorders has been permitted by the development of rapid and reliable immunofluorescence techniques. Immunofluorescence methods may also be used in monitoring of disease activity in some of the immunobullous disorders. Furthermore, these techniques have considerably advanced the understanding and classification of the immunobullous disorders. These techinques have been previously described.[1] The autoimmune blistering diseases may be subdivided into intraepidermal and subepidermal blistering disorders on the basis of the level at which blistering occurs. The direct and indirect immunofluorescence findings in each of the various disorders are described.

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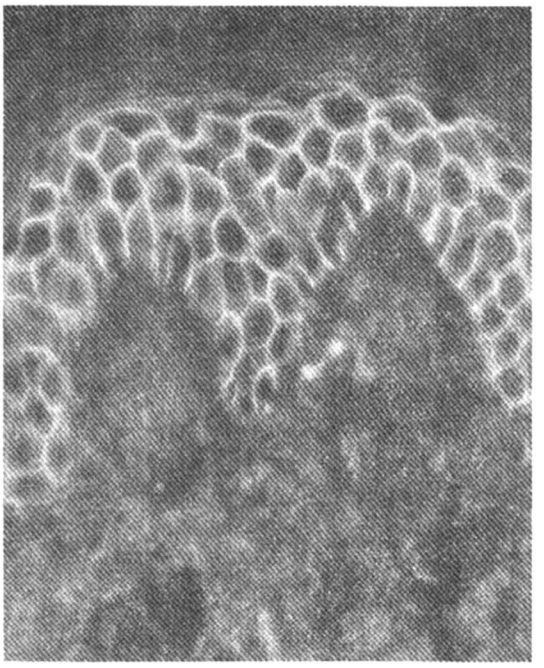
I. Intraepidermal Blistering Diseases

The pemphigus disorders are characterized by autoantibodies to desmosomal and other cell surface structures and result in intraepidermal blistering.

Pemphigus Vulgaris (PV)

Pemphigus vulgaris is characterized by thin-walled, flaccid bullae which rapidly rupture to leave slow-healing erosions. The site of onset is frequently in the mouth and other mucosae may also be involved. Biopsy findings are of separation at the suprabasilar level with acantholysis.[2]

Perilesional and clinically unaffected skin and mucosae has intercellular antibodies deposited throughout the epidermal intercellular substance (ICS) of almost all patients with active pemphigus (Fig 1).[3] IgG antibodies are usual but IgM, IgA and the C3 complement component are also present in approximately half of cases.[4] Direct immunofluorescence (DIF) may be used to assess disease activity during therapy-induced remission.[5] If negative, therapy may be ceased with a low risk of relapse but positive DIF is



associated with a high risk of relapsing should therapy be ceased.[5]

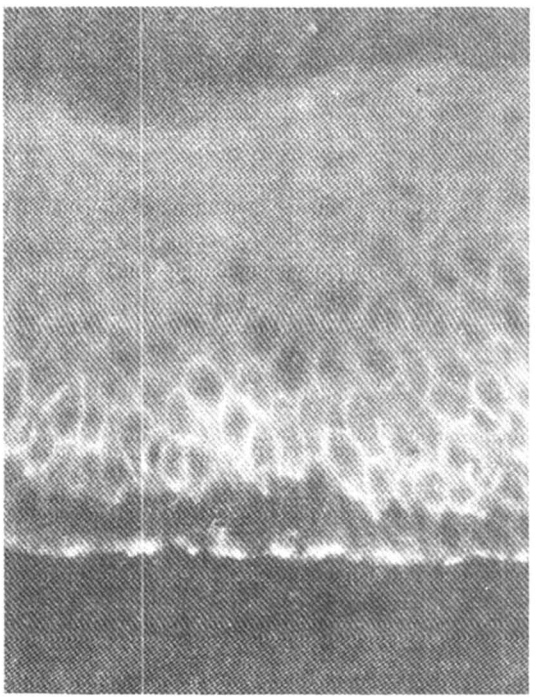
Indirect immunofluorescence (IIF) studies during active disease reveal an IgG antibody to the intercellular cement substance of stratified cornified epithelium in almost all cases.[6] The calcium enhancement technique will enhance detection of these antibodies whose titre reflects disease activity.[7,8] A two-fold or greater increase in the titre suggests impending relapse.[9] The absolute titre 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.[10] Antibodies are predominantly IgG4 subclass.[11,12] 'Pseudo-pemphigus' or anti-intercellular substance antibodies of low titre may be found in a variety of other conditions.

Pemphigus Foliaceus (PF) & Pemphigus Erythematosus (PE)

Blisters very rapidly rupture and are often not seen. Painful, crusted, offensive and inflamed erosions involve the central face, scalp, chest and upper back and may evolve into an erythroderma. Pemphigus erythematosus is a variant of pemphigus foliaceus with some clinical and immunological features of lupus erythematosus.[13] The nose and medial cheeks or 'butterfly' area of the face are affected by lesions as well as the usual sites.

Histology reveals acantholytic separation in the mid to high epidermis.[2]

Direct and indirect immunofluorescence findings are usually indistinguishable from those of pemghigus vulgaris although there may be preferential localization of immunoreactants to the upper epidermis. In pemphigus erythematosus immunoreactants are also frequently deposited in a granular pattern along the basement membrane zone, more commonly in sun-exposed areas.[14] This dual pattern of deposition is illustrated in Fig 2. Anti-nuclear antibodies are also often found.



IgA Pemphigus (IAP)

Flat pustules on an inflamed base have a tendency to confluence with the formation of annular and circinate patterns. Oral lesions are rare.[15] There is an association with benign and malignant monoclonal IgA gammopathies.[15]

A subcorneal pustule is usually present on biopsy but pustules at other levels in the epidermis and even subepidermally are sometimes present in addition or in isolation.[15]

By definition, intraepidermal IgA is present in all cases and may be found throughout the epidermis or restricted to the upper layers.[15] Other immunoreactants may occasionally be deposited in the intercellular substance and along the basement membrane zone.[15] Circulating IgA antibodies of low titre are present in more than half of cases.[15]

Paraneoplastic Pemphigus (PNP)

Paraneoplastic pemphigus is a specific paraneoplastic disorder, most commonly associated with lymphomas.[16] All mucosal surfaces may be affected by painful erosions. Cutaneous findings are highly variable and include target lesions on palms and soles as well as papules, vesicles and erosions on the trunk and limbs.[16]

Histology shows suprabasal acantholytic separation, satellite keratinocyte necrosis, basal cell vacuolation, spongiosis and epidermal exocytosis of inflammatory cell.[17,18]

IgG and C3 are almost invariably deposited in the intercellular substance in perilesional skin and mucosa.[16,19] C3, IgG and IgM may also be deposited along the basement membrane zone.[16,18,19]

Circulating IgG anti-intercellular substance antibodies are almost always present in high titre.[16] Rat bladder transitional cell epithelium is a specific and sensitive substrate for IIF in this disorder, giving an epithelial staining pattern.[20]

II. Subepidermal Blistering Diseases

Components of the basement membrane zone are the target of immunological attack in these disorders, giving rise to separation at the dermal-epidermal junction.

Bullous Pemphigoid (BP)

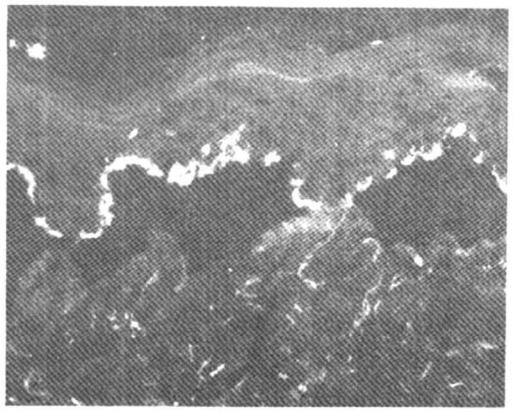
Prodromal urticarial and figurate erythemas are common. The large tense blisters arise on a base of normal or inflamed skin. Mucosal lesions are usually clinically insignificant.

Skin biopsies reveal subepidermal bullae with eosinophils. A mixed dermal infiltrate containing eosinophils is found.[2]

IgG and C3 are deposited in a thick linear band along the basement membrane zone (BMZ) in both perilesional and uninvolved skin of virtually all active cases of bullous pemphigoid.[21,22] Salt-splitting direct techniques enhance sensitivity of immunoreactant detection and help to differentiate between the various immunobullous disorders with immunoreactants deposited at the basement membrane zone.[23] IgG will localize to the roof of the split in the majority of patients, to both roof and floor in 10% but occasionally to the floor alone.[22,24] C3 will always bind to both roof and floor.[25] IgM, IgA, IgD and IgE may also be found at the dermo-epidermal junction.[21,22,26]

Circulating IgG antibodies may be found in 95% of cases by using salt-split skin as a substrate.[27] The antibodies are predominantly IgG1 and IgG4 subclasses.[28] Antibodies are usually lost in remission but fluctuations in the titre do not otherwise correlate with disease activity.[29] IgA and IgE antibodies may also be

found.[30,31] The use of salt-split skin substrate will usually reveal BP antisera binding to the epidermal side alone (Fig. 3) or to both epidermal and dermal aspects but occasional sera bind only to the dermal aspect.[32, 33] In such cases toad skin substrate may be utilized to confirm the diagnosis as it contains the bullous pemphigoid antigens but not those of epidermolysis bullosa acquisita.[33]

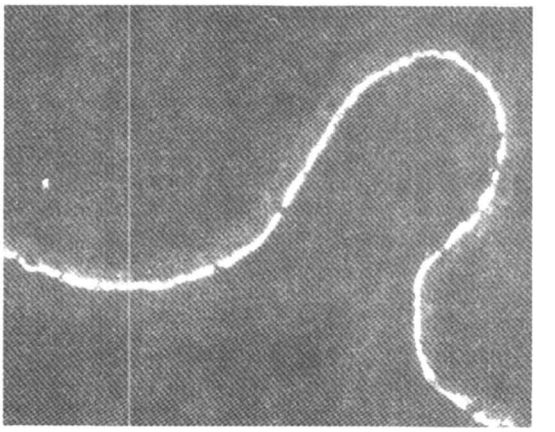


Pemphigoid (Herpes) Gestationis (PG)

Pemphigoid gestationis is a rare specific dermatosis of pregnancy. Initial lesions are usually in the periumbilical region and consist of pruritic urticated papules and plaques, target lesions and annular weals. Generalised involvement and the development of vesicles and bullae follows.

Biopsies of blistering lesions reveal eosinophil, lymphocyte and histiocyte-containing subepidermal bullae with eosinophil papillary microabscesses and a mixed infiltrate in the superficial dermis.[2]

The complement component C3 is deposited along the basement membrane zone (BMZ) in almost all active cases and IgG is also present in 30-40% of cases.[34] The detection of IgG may be increased with more sensitive multi-step procedures[35] or the use of labelled anti IgG1 rather than anti-IgG.[36] IgA and IgM may also be deposited at the BMZ.[37] IgG and C3 are found along the amniotic BMZ[36] (Fig. 4) and in the skin of the newborn.



The 'pemphighoid gestationis factor' is an IgG1 anti-BMZ antibody[38] and is detected in over 90% of cases by using the complement-binding IIF technique. The use of salt-split skin substrate will also greatly enhance sensitivity of IIF and binding is to the epidermal side.[36] The complement and salt-split skin techniques may be combined. The titre is not related to severity of disease nor duration.[39,40]

Cicatricial Pemphigoid (CP)

Mucosal and muco-cutaneous junctional involvement is prominent with painful, recurring and indolent blisters that heal with scarring and adhesions, complications of which include blindness and upper are digestive tract strictures. Cutaneous lesions occur in one fourth of cases and may heal with or without scarring.

- A subepidermal blister is found on biopsy and there is an evolving cellular infiltrate with phases of neutrophil, eosinophil and lymphocyte predominance.[2]

Perilesional skin and mucosae have linear deposition of IgG and C3 along the

BMZ in the great majority of active cases.[41,42] IgA and IgM are deposited less often.[41,42] Deposition of immunoreactants along the BMZ of mucosal mucous glands appears to be a specific finding in cicatricial pemphigoid.[43]

Circulating IgG and IgA antibodies are usually of low titre and are detected in 20-30% with standard IIF methods[44] but this may be increased to 80% by the use of salt-split skin substrate.[45] IgM antibodies may also be found.[46,47] 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.[27,45] IgG antibodies are usually IgG1 and IgG4 while IgA antibodies are always of IgA1 subclass.[47] Titres do not relate to disease extent nor activity.[41,46,47]

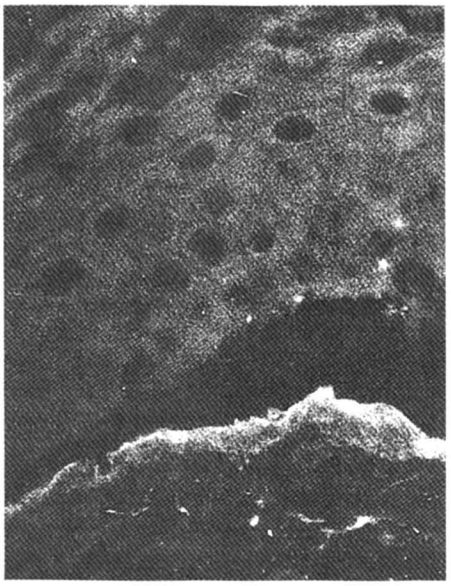
Epidermolysis Bullosa Acquisita (EBA)

Epidermolysis bullosa acquisita tends to begin with an inflammatory phase which may mimic bullous or cicatricial pemphigoid or dermatitis herpetiformis. The classic non-inflammatory mechano-bullous pattern of disease may follow or be the initial manifestation of disease. Minor trauma causes ulceration which heals with milia, scarring and hyperpigmentation. Mucosal involvement is common.[48]

A cell-poor subepidermal split with variable dermal cellular infiltration is seen on biopsy.[2]

IgG is deposited linearly along the BMZ of perilesional skin in all active cases.[48,49] IgA, IgM and C3 are also often present.[48,49] Salt-splitting DIF techniques reveal a dermal pattern of immunoreactant deposition in all cases (Fig. 6).[50] This floor pattern may rarely occur in BP and therefore does not reliably differentiate EBA from BP.[23,32]

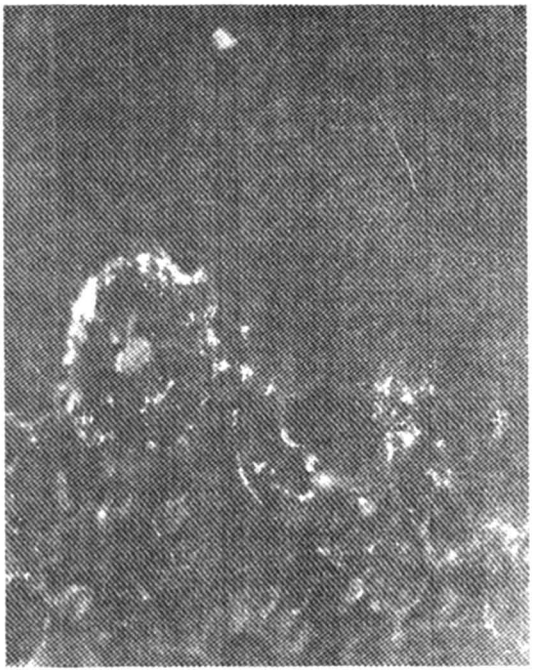
The detection and titre of circulating IgG anti-BMZ antibodies may be increased from 25-50% using standard IIF methods[48,49,51,52] to 50-85% with salt-split skin substrate.[24,53] A dermal pattern of binding is always obtained (Fig. 5) but is not entirely specific to EBA.[24] IIF studies performed using toad skin which contains the BP antigens but not those of EBA will be negative.[33] Circulating antibodies are more often found in the early inflammatory phase but the titre does not appear to otherwise correlate with disease activity.[54]



Bullous Systemic Lupus Erythematosus (BSLE)

Bullous systemic lupus erythematosus usually presents with a generalized eruption of tense vesicles and bullae with a non-inflamed base but may simulate bullous pemphigoid or dermatitis herpetiformis.[55] By definition, all patients should satisfy the American Rheumatological Association criteria for diagnosis of SLE.

The biopsy findings are similar to those of dermatitis herpetiformis with subepidermal separation and neutrophil papillary microabscesses.[2] Basal cell vacuolation, Civatte



bodies and dermal vasculitis are occasional findings.[2,55]

Direct immunofluorescence of perilesional skin shows IgG, IgM, IgA and C3 deposited along the dermo-epidermal junction, in the upper dermis and occasionally in small dermal venules.[56] IgG is always present and IgA and IgM are also frequently deposited.[56] The pattern of deposition may be granular (60%), linear (40%) or rarely fibrillar.[56] A linear rather than granular pattern along the BMZ is associated with the presence and higher titre of circulating autoantibodies.[55,56] 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.[54] C3 is usually deposited in lesional skin.[56]

The circulating antibodies are usually of low titre and the sensitivity of detection is increased with the use of salt-split skin substrate.[57,58] A dermal pattern is obtained with this technique. Immunoblotting studies may sometimes be positive when salt-split skin IIF is negative.[55] A high anti-nuclear factor titre may obscure positive BMZ fluorescence but removal of nuclear antigens from the substrate will permit its demonstration[58]

Bullous SLE may be divided into types I and II on the basis of the presence or absence of antibodies to type VII collagen.[56] Failure to demonstrate these antibodies with both IIF and direct immunoelectron microscopy permits classification as type II BSLE. The significance of this subclassification is unclear as clinical differences between the two types are not apparent.[56]

Dermatitis Herpetiformis (DH)

The extensor surfaces of the limbs, the buttocks, shoulders, axillary folds, face and scalp are affected by pruritic erythematous papules, urticarial weals and small vesicles, usually grouped on plaques of erythema. Gluten-sensitive enteropathy is present in all cases but may be subclinical in severity.

Biopsies show subepidermal vesicles which contain neutrophils, eosinophils and fibrin. Early lesions and perilesional skin show neutrophil papillary 'microabscesses'. There is a mid and upper dermal mixed perivascular infiltrate.[2]

Immunofluorescence studies on uninvolved skin show granular deposition of dimeric polyclonal IgA1 and C3 along the dermoepidermal junction, predominantly in the papillary dermis (Fig 6).[59] IgA is sometimes deposited in a linear-granular or fibrillar pattern.[60,61] The inflammation in lesional and perilesional skin often leads to removal of IgA deposits and these sites are therefore unsuitable for DIF studies.[62] A gluten-free diet may lead to clearance of IgA and C3 deposits as well as clinical improvement.[63,64] IgG and IgM occur in approximately 10% of cases and

may be linear or granular in distribution.[63,64]

Indirect immunofluorescence studies have been unable to demonstrate autoantibodies to skin antigens. Antibodies to endomysium of smooth muscle, gliadin, parietal cells and thyroid and positive antinuclear factors are common. IgA antiendomysial antibodies are specific to gluten-sensitive enteropathy, the titre correlates well with disease activity and thus may be used in dermatitis herpetiformis to monitor the underlying coeliac disease.[66]

Linear IgA Disease (LAD)

In children the disease often commences with urticated, annular to targetoid lesions with the subsequent development of the classic 'cluster of jewels' lesions of grouped small blisters around the edge of an erythematous annular lesion. Milia and scarring occur in the healing phase. Mucosal lesions are very common, particularly affecting the genitalia, and may also heal with scarring. Adults may present in a similar manner as children or with excoriated papulovesicles and prominent mucosal involvement. Lymphoproliferative diseases are increased in frequency.[67]

The histology is in many cases identical to that found in dermatitis herpetiformis but may resemble bullous pemphigoid.[2]

By definition, IgA is deposited as a linear band along the BMZ in all patients but initial false negatives may occur. Regional variation appears to exist with the forearm and conjunctiva being more often falsely negative.[68] Other immunoreactants (IgM, IgG and C3) are also present in 20-30% of patients.[69,70] The IgA deposits in LAD lack J chains[71] and are of the IgA1 subclass.[72] Epidermal, dermal and combined patterns of IgA deposition may be seen with salt-splitting of the biopsy.[73]

Children more commonly have positive indirect immunofluorescence than adults with figures of 72% and 20% respectively obtained in one study using standard techniques.[69] Salt-split skin substrate will increase the detection rate and titre of antibodies with binding to either the roof or floor, the former being more common.[74,75] Phenyl methyl sulphonyl fluoride must be added during the splitting process to preserve the epidermal antigen.[76]

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