Clinical Features and Diagnosis of Common Autoimmune Bullous Diseases in Hong Kong

Dr. PT Chan

MBBS(HK), MRCP (UK), FHKCP, FHKAM (Med)
Chai Wan Social Hygiene Clinic, Social Hygiene Service

Dr. PT Chan

The Spectrum of Autoimmune Bullous Diseases

Autoimmune bullous diseases are a group of cutaneous disorders characterised by skin blistering or erosions as a result of development of autoimmunity. It can be classified according to the anatomical sites of blister into two types: intraepithelial and subepidermal. It can be subdivided further according to the clinical features, type and location of immunoreactants found on skin biopsy (Table 1 and 2). The term "pemphigus" refers to intraepithelial blistering skin diseases. The term "pemphigoid" refers to blistering diseases occurring at the dermo-epidermal junction in general, although not every subepidermal bullous disease bears the term "pemphigoid" in the nomenclature (Table 2). With extensive research, most of the target antigens of these disorders have been characterised. It is amazing to see that earlier disease classification by clinical features and pathological findings alone did have a molecular basis, as distinct antigens are targets for different entities in most of the subgroup of autoimmune bullous diseases. The antigens of pemphigus are found in desmosomes between keratinocytes, which are organelles mediating intercellular adhesion together with tissue morphogenesis and differentiation. On the other hand, the antigens of subepidermal bullous diseases are found in the basement membrane zone at the dermo-epidermal junction. Research in these target antigens has led to a better understanding of their biologic functions, advance in disease diagnosis, monitoring of disease activity, and can be potentially employed for antigen specific therapy.

Table 1. Classification of major autoimmune intraepithelial bullous diseases and their target antigens

	Target antigen(s)
Pemphigus foliaceus	Desmoglein 1
Pemphigus vulgaris	Mucosal pemphigus vulgaris: Desmoglein 3 Mucocutaneous pemphigus vulgaris: Desmoglein 3 and 1
IgA pemphigus	Subcorneal pustular dermatosis type: Desmocollin 1 Intraepidermal neutrophilic type: Unknown
Paraneoplastic pemphigus	Desmogleins 1 and 3, Plakins, 170kD unknown antigen

Table 2. Classification of major autoimmune subepidermal bullous diseases and their target antigens

	Target antigen(s)
Bullous pemphigoid	BP180, BP230
Pemphigoid gestationis	BP180, BP230
Linear IgA bullous dermatosis	BP180
Epidermolysis bullosa acquisita	Collagen VII
Bullous lupus erythematosus	Collagen VII
Cicatricial pemphigoid	BP180, laminin, α_4 and β_6 subunits of integrin

Epidemiology of Autoimmune Bullous Diseases in Hong Kong

Autoimmune bullous diseases are uncommon in Hong Kong. In a local retrospective survey done in Social Hygiene Service in Hong Kong between 1985 and 1992, 234 Chinese patients were diagnosed by skin biopsies to have autoimmune bullous diseases.[1] Bullous pemphigoid (BP), pemphigus vulgaris (PV) and pemphigus foliaceus (PF) were the three most common autoimmune bullous diseases in Hong Kong and they accounted for 63.7%, 16.2% and 9.9% of cases respectively. BP tended to occur in the elderly whereas PV and PF affected patients with slightly younger ages. The mean age of presentation of BP patients was 70 year (standard deviation (SD) 15) and those for PV and PF were 57 year (SD 14) and 63 year (SD 13) respectively. These three entities are also the most characterised ones and the on going discussion will be concentrated on them.

Pemphigus Foliaceus

It can be divided into two major forms: sporadic and endemic types, although occasionally drug-induced PF is also seen. These two major forms have similar clinical lesions. Endemic PF is found in rural areas of Brazil, Columbia and Tunisia where clustering along rivers, within the same family, occurrence at young age and association with insect bite are observed.[2] Endemic PF provides an interesting model where an autoimmune disease arises as a result of interplay of genetic and environmental factors and there are dozens of papers published in this regard. PF manifests as cutaneous erosions. Intact blisters are less commonly found as the level of splitting is intraepidermal. Thus the roof of the blisters is relatively thin and easily ruptures. These erosions are commonly found in seborrhoeic areas, like the face, upper chest and back. Erythrodermic cases are sometimes seen, mainly in

patients suffering from endemic PF. Oral and mucosal erosions are not found in PF. The skin fragility in PF patients is demonstrated by the Nikolskiy sign. This sign indicates the ability to remove the superficial skin by pulling the edge of the cutaneous erosion or rubbing at the lesion edge and/or rubbing clinically the normal skin distant from the cutaneous erosions. There are variations of the tests such as the Asboe-Hansen sign; but these signs are not 100% specific as they only indicate skin fragility and can be found in other disorders as well, e.g. bullous pemphigoid, linear IgA bullous dermatosis and staphylococcal scalded skin syndrome.[3]

Pemphigus Vulgaris

PV, as opposed to PF, does have oral/ mucosal erosions. Similar to PF, as the roof of the blisters is thin, intact blisters are less commonly seen. It can be divided into two stages: mucosal PV and mucocutaneous PV. Patients with PV often presents initially as oral erosions, although other mucosal surfaces can also be involved (mucosal PV). About half of the patients will develop cutaneous erosions subsequently in addition to mucosal erosions (mucocutaneous PV). In severe forms, both PF and PV can disrupt the epidermal barrier leading to dehydration, electrolyte imbalance, temperature dysregulation, sepsis and can be life-threatening. Nikolskiy sign is also positive in PV.

Desmoglein: The Target Antigen in PF and PV

Desmoglein 1 as a common target in PF and staphylococcal scalded skin syndrome

The target of autoimmunity in PF is desmoglein 1. It is a transmembrane glycoprotein located in the desmosomes. Desmoglein 1 is mostly found in the subcorneal layer of the epidermis and this is the level of split observed on histopathology (see Diagnosis of autoimmune bullous diseases). Similar subcorneal splitting in the epidermis is also observed in staphylococcal scalded skin syndrome and bullous impetigo. The reasons of their similarity remained puzzle for a long time until it was found out that the exfoliative toxin of *Staphylococcus aureus* was a serine protease that cleaved desmoglein 1. Thus, both PF and staphylococcal scalded skin syndrome are diseases secondary to loss of desmoglein 1 function by autoantibodies and bacterial enzymes respectively.[4] On body surface, most desmoglein 1 is localised in the seborrhoeic areas where PF lesions are often found.[5]

Desmoglein compensation hypothesis

The autoimmune target of mucosal PV is desmoglein 3. It is hypothesised that the phenomenon of epitope spreading occurs subsequently, leading to the development of autoimmunity to both desmogleins 1 and 3 in mucocutaneous PV. Subsequent in vivo studies have shown that desmogleins can compensate for the function of each other.[4] In normal human epidermis, desmoglein 1 is expressed mainly on the subcorneal layer, with some extension below to suprabasal areas; whereas desmoglein 3 is mainly expressed on the suprabasal layers, not extending to subcorneal layers. Thus in PF, the loss of desmoglein 1 function by anti-desmoglein 1 antibodies would lead to subcorneal splitting on skin surface, where only desmoglein 1 is found.

In normal human mucosa, desmoglein 1 is also expressed subcorneally and desmoglein 3 is expressed mostly suprabasally. In contrast with skin epithelium, the expression of desmoglein 3 extends to the subcorneal layer in mucosal areas and desmoglein 1 expression is only limited to subcorneal layers without further extension. In PF, no mucosal lesions are observed as the presence of desmoglein 3 across the entire mucosal epithelium compensates for loss of desmoglein 1 function.

In mucosal PV, where anti-desmoglein 3 antibodies are found, suprabasal splitting occurs at mucosal surface only as this site only expresses desmoglein 3 without desmoglein 1. In skin epithelium, the presence of some desmoglein 1 in suprabasal layers compensates for loss of function of desmoglein 3 and no skin blistering is observed. But when anti-desmoglein 1 and 3 antibodies are present in mucocutaneous PV, suprabasal blisters occur both in the mucosa and skin surface. This hypothesis is a good model illustrating how basic science can help physicians to understand the pathophysiological basis of clinical disease phenotype observed in their practice.[6]

Bullous Pemphigoid - Clinical Features and its Immune Target

BP is the most common autoimmune bullous diseases in Hong Kong. It often affects elderly patients. Patients with BP typically present as intact blisters as the roof of blister, which is comprised of the entire epithelium, is thicker than that in pemphigus. The blister size ranges from small to large and can develop on erythematous, urticarial or even normal looking skin. Blister fluid can be clear or haemorrhagic. These blisters are most commonly found over the flexural areas of skin surface, such as the abdomen, inner thighs, groins or axillae but they can occur everywhere. Classically, no scarring or milia are seen in BP, as opposed to diseases affecting deeper part of dermo-epidermal junction such as epidermolysis bullosa acquisita. Mucosal surfaces are affected in 10-40% of cases. But as buccal cavity is a confined space, blisters readily rupture in this area and erosions are seen instead. Usually patients with BP suffer from intense pruritus. Sometimes patients with BP present atypically as pruritic nodules (pemphigoid nodularis), localised blisters over palms or soles (dyshidrotic BP), figurate urticarial lesions or vulval erosions especially in children. A high index of suspicion is required for diagnosing these atypical BP.

The target antigens of BP are BP180 and BP230.[7] BP180 is a transmembrane protein found in the lamina lucida of the basement membrane zone. BP180 is a large molecule and within the molecule, an extracellular non-collagenous domain, NC16a, is found to be an important autoimmune epitope in BP. On the other hand, BP230 is a molecule located intracellularly in hemidesmosomes, an organelle important for adhesion of basal cells to basement membrane. Thus the site of detachment in BP is at the basement membrane and subepidermal blister is found on histology (see Diagnosis of autoimmune bullous diseases).

Diagnosis of Autoimmune Bullous Diseases

The diagnosis of autoimmune bullous disease requires clinico-pathological correlation. An exhaustive list of differential diagnosis of skin blisters or erosions is beyond the scope of this short review, but potential ones include inherited epidermolysis bullosa, herpes simplex/ zoster, staphylococcal scalded skin syndrome, bullous impetigo, lichen planus pemphigoides, scald injury, fixed drug eruption, toxic epidermal necrolysis, porphyria cutanea tarda, diabetic bullae, pseudoporphyria, etc. It is essential to perform skin biopsy for histopathology and direct immunofluorescence test (DIF) to establish a firm diagnosis, bearing in mind that the treatment of these diseases often requires long term treatment with immunosuppressant.

In PV and PF, the core features include intraepithelial blisters/ split, acantholysis (which signifies loss of cellular adhesion) and variable underlying dermal inflammatory infiltrate. In PF, the split is more superficial (subcorneal) whereas in PV, the split is deeper down (suprabasal). In BP, subepidermal blisters are observed and the typical inflammatory infiltrate is predominantly eosinophilic. Besides, secondary changes such as scale crust formation or re-epithelialisation may be present depending on the stage of evolution.

Direct Immunofluorescence Test

DIF detects immunoreactants present on biopsied tissues and by definition all autoimmune diseases should have positive DIF to immunoglobulin. However, occasionally, errors in choosing the biopsy site, previous treatment with topical or oral steroid or delay in transport of specimen may lead to false negative. In PF/ PV, the IgG are bound to keratinocyte intercellular surfaces, while in BP, IgG are bound to the basement membrane zone as a linear band. Complement is found in almost all BP cases and may be variably found in PV or PF on DIF.

Indirect Immunofluorescence Test

The historical landmark finding of the presence of circulating anti-skin antibodies in sera of pemphigus patients first defined it as an autoimmune disease.[8] Then it was observed that titre of anti-skin antibodies correlates with disease activity in pemphigus, but not in BP.[9] Titre of anti-skin antibodies is conventionally estimated by the indirect immunofluorescence test (IIF). The basic principle of this test is to determine the highest sera dilution that can still give a positive intercellular surface (in PV or PF) / linear basement membrane (in BP) staining pattern on a predefined epithelial substrate as determined by immunofluorescence microscopy. The titres are often represented as multiples of a fraction, like 1/10, 1/40 or 1/160. However, the test is both operator and substrate dependent.

With the identification of target antigen and advancement of molecular biology, these antigens can be produced and purified in vivo. Now commercial ELISA kits are available for measuring desmoglein 1 and 3 reactivity.[10] Besides being sensitive and specific, these ELISA kits have several advantages over the conventional IIF in that they are more objective, antibody titre is represented by continuous valuables and they are not substrate dependent. Desmoglein 1 and 3 ELISA indexes have been proven to correlate well with disease activity in PF and PV. The titre of antibody in BP, as measured by IIF, does not correlate with disease activity. By the development of separate ELISA kits for BP180 and BP230, it has been found out that ELISA BP180 index does correlate with disease activity but ELISA BP230 index only sometimes fluctuates with change in disease activity.[7, 11] As titre of antibodies measured by IIF represents both anti-BP180 and anti-BP230 activities, it explains why older studies have failed to demonstrate a relationship between antibody titre and disease activity in BP.

ELISA tests reflect the amount of antibodies in patients' sera but not the presence of antibodies bound to skin of the patients. To diagnose immunobullous disease by skin biopsy, besides the level of splitting/ blister, we aim at finding the presence of tissue bound immunoglobulin by DIF as a circumstantial proof of its aetiology of skin blistering. There is some degree of overlap of desmoglein 1 and 3 ELISA indexes in pemphigus patients with those in patients suffering from BP and other connective tissue diseases, especially at marginally elevated ELISA indexes.[12] Moreover, commercial ELISA kits are not available for some antigens of autoimmune bullous skin diseases, such as desmocollin 1 and plakins. Thus, ELISA test cannot replace DIF completely as a diagnostic test of autoimmune bullous diseases.

Conclusion

In summary, BP, PV and PF are the three most common autoimmune bullous diseases seen in Hong Kong (comparison summarised in Table 3). The diagnosis of autoimmune skin diseases requires clinico-pathological correlation. Although newer ELISA kits are now available, they play a role in monitoring antibody titre but cannot completely replace DIF of biopsied skin in diagnosis of various autoimmune bullous diseases.

Table 3. Comparison of clinical and histological findings among pemphigus foliaceus, pemphigus vulgaris and bullous pemphigoid

	Pemphigus foliaceus	Pemphigus vulgaris	Bullous pemphigoid
Lesion morphology	Erosions +/- crusting; intact blisters rarely seen	Erosions +/- crusting; intact blisters rarely seen	Intact blisters common; erosions seen only if blister ruptures
Distribution	Commonly found on seborrhoeic areas (face, upper trunk); no mucosal lesions seen	Mucosal erosions common as initial presentations, on progression both mucosal and cutaneous lesions develop	Flexural surfaces such as abdominal wall, groin or axilla, but can affect everywhere; mucosa may be involved in 10-40% of cases
Major histological findings	Subcorneal splitting, acantholysis	Suprabasal splitting, acantholysis	Subepidermal blisters, eosinophilic infiltration
Direct immunofluorescence	Intercellular IgG staining; C3 staining variably present	Intercellular IgG staining; C3 staining variably present	Linear basement membrane IgG + C3 staining
Correlation of antibody level (estimated by indirect immunofluorescence test) with disease activity	Present	Present	Absent

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