Immunodiagnosis of pemphigus and mucous membrane pemphigoid

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Pemphigus and pemphigoid are two of a group of bullous diseases affecting oral mucosa and skin. Mucous membrane pemphigoid (MMP) comprises a heterogeneous group of disorders characterized by subepithelial separation and the deposition of immunoglobulins and complement along the basement membrane zone (BMZ). The target antigens in the epithelium and BMZ determine the nature of the condition, and recently there have been considerable improvements in our understanding of the BMZ antigenic composition. Pemphigus vulgaris (PV) is characterized by autoantibodies of the IgG isotype to the desmosomal glycoprotein desmoglein (Dsg) 3, whereas pemphigus foliaceus targets Dsg1, although at least 50% of PV patients have additional autoantibodies to Dsg1. The clinical phenotype appears to be determined by the relative amounts of Dsg1 and Dsg3. Patients with oral or mucosal PV have predominantly Dsg3 autoantibodies. The most frequently targeted antigen in MMP is bullous pemphigoid antigen 180 (BP180), although bullous pemphigoid antigen 230 (BP230), laminin 5, and beta 4 integrin are also involved. Circulating IgG and IgA antibodies may bind to different epitopes of BP180—namely the NC16A domain or COOH-terminal domain. Pure ocular disease has been associated with IgA antibodies to a 45-kDa antigen and IgG antibodies to the 205-kDa antigen b4 integrin. The use of salt-split skin substrate enables differentiation between epidermal and dermal 'binders'. Since both the specificity and the antibody titer appear to have direct relationships with the disease severity, and a combination of clinical score and antibody titer provides valuable prognostic data, these investigations should be carried out on a more routine basis. □ *Autoantibodies; basement membrane; desmogleins; mucous membrane*

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Pemphigus and pemphigoid are two of a group of bullous diseases affecting the oral mucosa and skin and which include linear IgA disease, epidermolysis bullosa aquisita, and systemic lupus erythematosus. They are both autoantibody-mediated diseases, although the target antigens are quite different in type and location. Mucous membrane pemphigoid (MMP) comprises a heterogeneous group of disorders characterized by subepithelial separation and the deposition of immunoglobulins and complement along the basement membrane zone (BMZ), whereas pemphigus is characterized by clefting and acantholysis within the epithelium owing to the binding of IgG autoantibodies to desmogleins (see below).

MMP affects from 2 to 5 patients per 100,000 (1), and more than 85% of patients have oral involvement (2, 3). Pemphigus affects between 0.1 and 0.5 patients per 100,000 population per year, depending on the population studied (4). Oral lesions of pemphigus have been reported in up to 18% of patients at dermatology outpatient clinics (5).

Epithelial and basement membrane antigens

The oral epithelium is a complex structure consisting of a range of cells, mainly keratinocytes, adherent to each other by desmosomes, and via hemidesmosomes and an epithelial basement membrane to the underlying lamina propria. Each component is itself complex and consists of several proteins with important functions—not least the adherence of cells to adjacent structures and cell–cell recognition and signaling (Fig. 1). The basement membrane zone comprises the basal cell plasma membrane, the lamina lucida, the lamina densa, and the sublamina densa. On the ventral surface of basal keratinocytes are small electron-dense domains of the plasma membrane, the hemidesmosomes (HD). These are specialized junctional complexes that contribute to the attachment of epithelial cells to the underlying basement membrane. Several proteins implicated in the pathogenesis of subepidermal blistering diseases are associated with the HD and include the bullous pemphigoid antigens BP230 and BP180 and the $\alpha 6\beta 4$ integrin (6). The lamina lucida is the electron-lucent zone adjacent to the basal cell plasma membrane and is 20–40 nm thick. Anchoring filaments transverse the lamina lucida perpendicularly from the basal cell membrane to the underlying lamina densa. Together with HD they form the HD anchoring filament complex, which has an important role in cell-basement membrane adhesion. The lamina densa is the electron-dense layer below the lamina lucida and is thought to be thinner in female than in male skin and possibly mucosal epithelium.

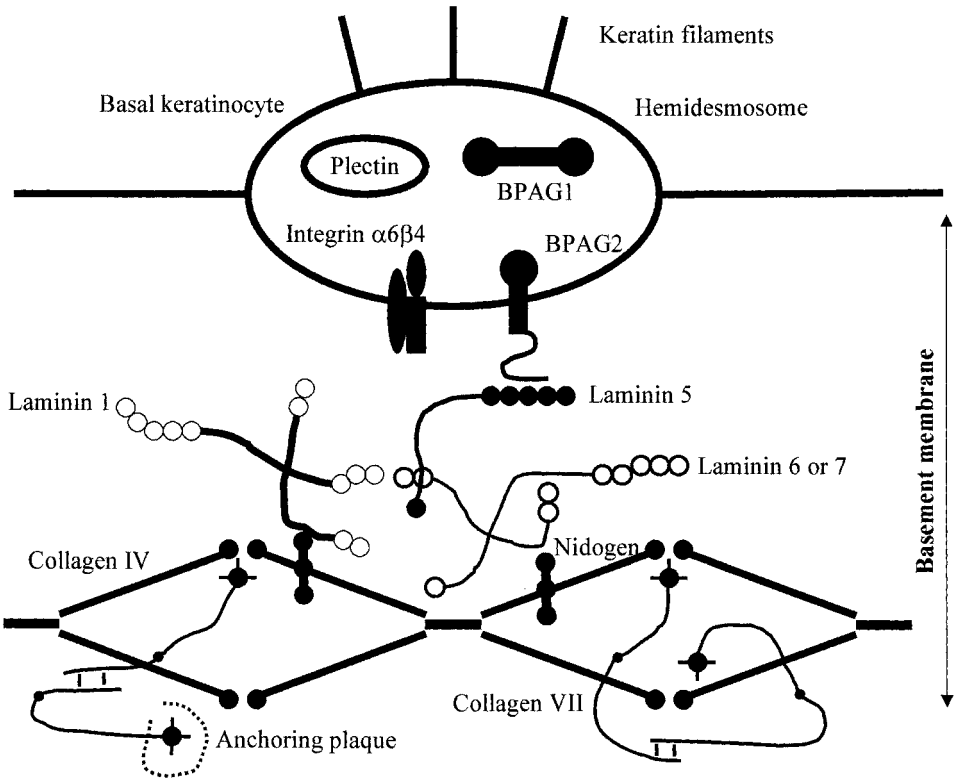

Fig. 1. Structural components of the basement membrane zone.

Epithelial cell–cell contact is via occludens (tight junctions), adherens (desmosomes and adhesion plaques), and nexus junctions (gap junctions), each having a complex structure. Desmosomes guarantee the integrity of the epithelium by functioning both as an adhesive complex and as a cell surface attachment site for the keratin intermediate filaments of the cytoskeleton. Desmosomes are adhesion proteins that contain a series of proteins, particularly desmogleins and desmocollins—glycoproteins of the cadherin family that link to cytokeratins via desmoplakins and plakoglobin (7).

Oral epithelium is closely similar to skin but differs in several essentials; for example, desmoglein 1 (Dsg1) and Dsg3 are expressed in skin, but oral epithelium expresses predominantly Dsg3 (8). This has consequences in terms of disease manifestations, as discussed below, and in antibody detection.

The BMZ contains a mixture of structural components and antigens including type-VII collagen, which is the major structural component of anchoring fibrils, and type-IV collagen, which is a major component of vertebrate basement membranes and shows diversity in its subunit composition. All the alpha chains contain three primary regions: an amino-terminal 7S domain, a central triple helical portion interrupted in several areas by non-helical segments that provide the molecule with flexibility, and the collagenase-resistant carboxy-terminal globular domain (NC1), which contains 12 highly conserved cysteine residues.

Laminins are the most abundant non-collagenous glycoproteins of basement membranes and exist in a wide variety of molecular forms. They are composed of genetically distinct alpha, beta, and gamma chains, and 11 laminins are now recognized. Entactin (nidogen) is a 150-kDa glycoprotein interacting with both laminin and type-IV collagen. In addition, there are several heparan sulfate proteoglycans, with molecular weights up to 400 or 500 kDa. The pemphigoid antigens are protein antigens, the main ones being 230 and 180 kDa, although several others have been described (see below).

BP230, also known as BPAG1, is localized at the inner plate of the HD, whereas BP180 is a transmembrane molecule and part of the HD anchoring filament complex (9). An immunodominant antigenic site within the extracellular non-collagenous domain of this protein called NC16A is recognized by serum from most pemphigoid patients. The $\alpha 6\beta 4$ integrin is a further transmembrane constituent of the HD which binds with high affinity to laminin 5, whereas the $\alpha 6$ subunit is thought to interact with the NC16A domain of BP180 (10).

The epithelium thus has a complex structure, and an array of molecules is required for epithelial integrity and health (Fig. 1) (11).

Mucous membrane pemphigoid

Mucous membrane pemphigoid consists of a group of subepithelial blistering diseases primarily involving mucosal surfaces and occasionally the skin. Mucous membrane pemphigoid (MMP) is now the preferred term when the condition is primarily mucosal, and bullous pemphigoid (BP) when the condition primarily involves the skin.

Clinical features

The disease typically presents in patients between 55 and 70 years of age, although childhood cases have been reported (12). The mean age found in a literary review of 457 patients was 62 years (2). The female to male ratio is approximately 2:1.

Oral lesions are the most common presentation and can be found in 85% to 90% of patients (3, 13). Virtually all sites in the oral cavity can be involved, including gingivae, buccal mucosa, palate, and, less commonly, alveolar ridges, tongue, and lips. A subgroup of patients presents with desquamative gingivitis (DG) as the sole manifestation, although other patients present with involvement of parts of the oral mucosa without DG, and in a third group all of the oral mucosa can be involved.

Ocular lesions

The conjunctiva appears to be the second most frequent site of involvement and in a clinical subgroup is often the only site affected. Conjunctival biopsy specimens from patients presenting with oral disease show basement membrane zone deposition in more than 50% of patients (3); this strongly suggests that routine ophthalmological examination should be part of the clinical protocol for patients presenting with oral ulceration. Most cases of clinical ocular presentation are bilateral, although the disease often begins unilaterally and progresses to the other eye over several years (14). Ocular MMP begins as a non-specific chronic conjunctivitis leading progressively to subconjunctival fibrosis with symblepharon formation and forniceal shortening.

Skin lesions

Two types of skin lesions are evident, the first type consisting of recurrent vesicles and bullae, which rupture and heal without significant scarring (15). The second type is known as the Brunsting Perry variant, in which lesions are predominantly localized to the head and neck and consist of flaccid blisters that, after ulceration, heal slowly and leave atrophic scars. On the scalp, recurrent blistering may result in cicatricial alopecia (16).

Other sites

In our series of 67 patients (3), now extended to 140, involvement of the nasopharynx was found in almost one-third of cases, the genitalia in 28%, esophagus in 7.5%, and larynx in 5%.

Nasal involvement usually consists of a crusted ulceration on the septum or turbinates (17). Pharyngeal involvement is typically manifested by ulceration of the posterior or lateral pharynx and presents with dysphagia. Involvement of the larynx is often clinically manifest as hoarseness, and laryngeal erosions and adhesions may be seen on examination (17).

Vesicles and erosions of the labia majora or minora in women and of the glans penis and foreskin in men can lead to fibrosis and adhesion formation (1). Complications include urethral stenosis and narrowing of the vaginal orifice, and scarring in these areas may require surgical intervention (18). Anal vesicles and erosions can also lead to scarring and stricture formation (1).

Human leukocyte antigen associations in MMP

Most studies have looked at relatively small numbers of patients, and the earliest reports were contradictory. More recent studies have shown an association between ocular cicatricial pemphigoid (OCP) and human leukocyte antigen (HLA)-DR4, -DR5, and -DQw3 (19, 20). MMP as a whole has been associated with HLA-DR4 and DQw3 (21). Since then, the HLA-DQw3 has been subtyped as the DQw7 (DQB1*0301) allele by using restriction fragment length polymorphism in genomic DNA (22). In our own series of 130 patients (23) HLA-DQB1*0301 was shown to be associated with all clinical sites of involvement, extending the earlier association with oral and ocular subgroups (24). No associations with Class I HLA have been confirmed.

Class II DRB1 typing showed a significantly increased allelic frequency in MMP compared with controls for DRB1*11 (23). Interestingly, the DQB1*0301 allele was significantly increased in patients with detectable circulating anti-BMZ IgG, suggesting that this allele may be linked to the anti-basement membrane IgG production.

Immunofluorescence

Direct. Direct immunofluorescence (DIF) investigations using perilesional mucosa show a linear continuous band at the BMZ, usually with IgG and C3 but often with IgA in virtually 100% of patients with the clinical characteristics of MMP (24). DIF is essential for the diagnosis of MMP and must be performed to complement the clinical findings.

Indirect. Early studies either failed to show circulating antibodies or only a small percentage of patients with circulating IgG antibodies (25). Subsequent studies (3) showed that circulating IgG anti-BMZ antibodies could be

Table 1. Basement membrane zone target antigens in mucous membrane pemphigoid subgroups

Antigen	Disease type*	Ref. no.
BP 180	MMP, BP	28
BP 230	BP, MMP	27
120 kDa	BP, MMP, LAD	72
Laminin-5 (epiligrin)	Subgroup MMP	30
	Paraneoplastic MMP	38
Laminin-6	Subgroup MMP	29
p105	?	31
45 kDa	Ocular	41
Beta 4 integrin	Ocular	
168 kDa	Oral	73
LABD 97 kDa	Linear IgA disease	74

* MMP = mucous membrane pemphigoid; BP = bullous pemphigoid; LAD = linear IgA disease.

detected in more than 80% of patients and IgA in more than 50%. The increased detection rate may be linked to the substrate use for these studies, since salt-split skin was shown to be significantly better than intact skin, oral mucosa, or rabbit esophagus, particularly with regard to IgA, for which the detection rate increased from less than 20% on intact skin to more than 50% on salt-split skin.

It has now been shown that the dual antibody response with IgG and IgA signifies a more severe and persistent disease and that there is a significant relationship between the titer of IgG and the presence of IgA with the severity of the disease (26). This observation strongly suggests that investigation of patients with MMP should include indirect immunofluorescence using salt-split skin as a substrate on a routine basis. Most patients have antibody binding to the roof of salt-split skin, but a subgroup has binding to the floor, which may be associated with anti-laminin-5 antibodies. An analysis of IgG subclasses has shown IgG1 and IgG4 as the predominant subclasses but only IgG1 associated with complement deposition (27).

Target antigens

MMP comprises a group of disorders with a wide spectrum of clinical presentation. It is therefore not surprising that the literature indicates a wide variation of target antigens involved in the BMZ. The main characterized antigens are the bullous pemphigoid antigens (BP180 and BP230) (28, 29), laminin 5 (30), laminin 6 (31), and beta 4 integrin.

Table 2. Clinical subgroups of mucous membrane pemphigoid with immunoglobulin isotypes and main target antigens

Site	Clinical subgroup	Isotype	Main target antigens
Skin	BP*	IgG ++	180,230 kDa
	Uncommon	IgA ++	180 kDa
Ocular only	MMP†	IgA ++	45 kDa
	Uncommon	IgG +	Beta 4 integrin
Oral	MMP	IgG ++	168,180,230 kDa
	Common	IgA +	180 kDa
Skin and oral	MMP	IgG ++	180,230 kDa
	Common	IgA ++	180 kDa

* BP = bullous pemphigoid.
† MMP = mucous membrane pemphigoid.

BP180 is the antigen most frequently associated with MMP (Table 1). At least two antigenic sites on the extracellular domain have been identified (32); one is located proximally, close to the membrane spanning region, the NC16A domain, and the other is located near the carboxy terminus. Although sera from patients with MMP or BP may react with BP180, the NC16A domain is favored by BP, whereas either or both sites may be recognized in MMP. Ultrastructural studies in MMP have shown that antibodies appear to be localized to the lower lamina lucida and lamina densa, whereas in BP antibodies against BP180 seem to be in the upper lamina lucida (33). IgA autoantibodies to BP180 appear to react against different epitopes than IgG antibodies (34).

Laminin 5 is an adhesion molecule consisting of alpha 3, beta 3, and gamma 2 subunits and is localized to the anchoring filaments within the lamina lucida. IgG anti-BMZ autoantibodies show reactivity to the subproducts of 170, 145, 125, and 95 kDa. Laminin 5 and laminin 6 share the same alpha 3 subunit, and it is likely that they are co-targets for some autoantibodies. Patients with anti-laminin-5 cicatricial pemphigoid frequently have a severe disease phenotype with multi-site involvement. Interestingly, an association between anti-laminin 5 CP and an underlying malignancy is now recognized (35–37), and in at least one case the criteria for paraneoplastic disease were fulfilled (38).

The beta 4 integrin subunit is associated with the alpha 6 subunit, and this complex functions as an integral part of the epidermal HD. Patients with ocular pemphigoid may develop IgG antibodies to a 205-kDa protein that appears to be part of the beta 4 integrin complex (39).

Recent immunopathological and immunochemical techniques have shown that MMP consists of several distinct subgroups based on the specificity of antibodies (Table 2). The largest subgroup targets BP180 (27, 31–33). A further group has circulating IgG antibodies that bind to the dermal side of salt-split skin and recognize laminin 5 (previously known as epiligrin) (40). This group may also have antibodies directed against laminin 6. Among the clinical subgroup with pure ocular disease, IgA antibodies may target an uncharacterized keratinocyte-derived 45-kDa antigen (41), and IgG antibodies may target epitopes on beta 4 integrin (39).

Pemphigus

Pemphigus is a group of autoimmune disorders in which there is damage to desmosomes by antibodies directed against cadherin-type epithelial cell adhesion molecules—desmogleins (Dsg)—(42), with immune deposits intra-epithelially and loss of cell–cell contact (acantholysis),

Table 3. Main types of pemphigus involving the mouth and their putative antigen targets

Pemphigus variant	Oral lesions	Main antigens	Localization of antigens	Antibodies	Ref. no.
Pemphigus vulgaris localized to mucosae (mucosal)	Common	Dsg3	Desmosomes	IgG	43, 47, 60
Pemphigus vulgaris also involving skin/ other mucosae (mucocutaneous)	Common	Dsg3 Dsg1	Desmosomes	IgG	43, 47
Paraneoplastic pemphigus	Common	Desmoplakin 1 Desmoplakin 2 BP 230, 170, 190 kDa 210, 230 kDa 250 kDa Periplakin	Desmosomes or hemidesmosomes	IgG or IgA	75
Drug-induced pemphigus	Common	Dsg3	Desmosomes	IgG	77
Pemphigus foliaceus	Very uncommon	Dsg1	Desmosomes	IgG	60, 76
IgA pemphigus	Very uncommon	Desmocollin 1 Desmocollin 2 Dsg3	Desmosomes	IgA	78

leading to intraepithelial vesiculation. Pemphigus affects the skin and may also affect the mucosae of the mouth, nose, conjunctivae, genitals, esophagus, pharynx, and larynx and is found mainly in middle-aged and elderly patients.

Several variants of pemphigus have been described (Table 3), with different autoantibody profiles and clinical manifestations; for example, the main antigen in pemphigus vulgaris (PV) is Dsg3 (43, 44), whereas that in pemphigus foliaceus is Dsg1 (43). However, 50% of PV patients also have autoantibodies to Dsg1, and the proportion of Dsg1 and Dsg3 antibodies appears to be related to the clinical severity (45, 46). Those PV cases that are predominantly oral have only Dsg3 antibodies (47, 48) (Table 4).

PV is the most common form and frequently involves the mouth (49, 50). In contrast, other types of Pemphigus, such as pemphigus foliaceus (PF) and erythematosus and pemphigus vegetans, only rarely affect the oral mucosae (4, 51).

Apart from PV, the other important form affecting the oral mucosa is paraneoplastic pemphigus, usually associated with lymphoproliferative disease (52), although one case with oral squamous carcinoma has been reported (53). Oral lesions have also been seen in all reported cases of paraneoplastic pemphigus (54, 55) and may be the sole manifestation (56).

Table 4. Antibodies to desmogleins in pemphigus

Pemphigus variant	Localization	Dsg3	Dsg1
Vulgaris	Mucosal mainly	+	–
Vulgaris	Mucocutaneous	+	+
Foliaceus	Cutaneous	–	+

After Harman et al. (47).

HLA associations

Dsg1 autoantibodies are found in more than 50% of cases of PV, and the frequency may differ with race, since they are found in a significantly greater proportion of patients of Indian origin than white northern Europeans (45). Dsg1 antibodies in the presence of Dsg3 antibodies are associated with a more severe phenotype of PV (46). PV IgG subclasses are detectable not only in patients but also in their first-degree relatives (57).

HLA class-II allele associations in PV are found with HLA-DR4 (DRB1*0402), DRw14 (DRB1*1041), and DQB1*0503 (58–60). These HLA class-II alleles appear to be critical for T-lymphocyte recognition of Dsg 3 peptides. Two kinds of Dsg 3-derived peptides may be presented by HLA-DR in accordance with the HLA polymorphism (DRB1*0402 or DRB1*14/0406). The DRB1*14/0406 PV-related molecules may be able to present Dsg1 and Dsg3 peptides, providing one explanation for cases of PV with combined responses to Dsg1 and to Dsg3, which are typified by a mucocutaneous clinical phenotype (61).

Pathogenesis

Any of the desmosomal proteins can be defective or damaged, and this can result in loss of cell–cell adhesion leading to the clinical result of vesiculation, erosions, or ulcers, which characterize pemphigus. PV is an autoimmune disorder in which there is damage to desmosomes by antibodies directed against cadherin-type epithelial cell adhesion molecules, particularly Dsg3 (42). Since oral epithelium expresses largely Dsg3, but skin expresses both Dsg1 and Dsg3, damage by antibodies to Dsg3 as in PV results in oral lesions at an early stage, whereas skin integrity is maintained by Dsg1; however, if Dsg1 antibodies appear, cutaneous lesions appear to result,

and the disease is more severe (47) (Table 3). The Dsg autoantibodies in active PV are predominantly IgG4 polyclonal antibodies, but IgG1 while in remission (39).

There is direct evidence that autoantibodies against Dsg3 are critical in the pathogenesis, since the transfer of PV serum IgG antibodies against Dsg3 into newborn mice induces a bullous skin disease resembling PV (42, 56), and rDsg 1 and rDsg 3 absorb the antibodies that cause PV-like skin blisters in neonatal mice.

The precise mechanism of the acantholysis after pemphigus IgG binds to Dsg3 on the cell surface is unknown. PV IgG causes a transient increase in intracellular calcium and inositol 1,4,5-trisphosphate concentration and subsequent activation of protein kinase C (PKC) in cell lines. The phosphatidylcholine (PC)-specific phospholipase C (PLC) pathway plays a major role in P-IgG-induced transmembrane signaling by causing long-term activation of PKC (62). Plasminogen activation and apoptosis may also be involved. The appearance of antibodies to Dsg1 in PV correlates with disease progression and severity (46, 47).

Antigens other than desmogleins

Non-desmoglein antibodies have been shown to induce pemphigus-like lesions in neonatal mice, causing gross skin blisters with PV-like suprabasal acantholysis and staining perilesional epithelium in a fishnet-like pattern. This indicates that the PV phenotype can be induced without anti-Dsg3 or anti-Dsg1 antibody (63).

Cellular immunity in PV

Although the PV autoantibodies are pathogenic, the role of the cellular immune system in acantholysis is unclear. There is a sparse cellular infiltrate at the BMZ. However, autoreactive T-cell responses to Dsg3 may be critical to the pathogenesis, since antibody production generally requires T-cell help; the strong association with distinct HLA class-II alleles (see above) suggests the involvement of CD4+ T lymphocytes. These T cells recognize epitopes of Dsg3. Most of the T cells are CD45RO (64) and help autoreactive B lymphocytes to produce autoantibodies. CD28-deficient mice (lacking a co-stimulatory signal for T lymphocyte activation) are much more sensitive to the development of PV than wild-type mice (65). T-cell recognition of epitopes of Dsg3 may be crucial for the initiation and perpetuation of the production of Dsg3-specific autoantibodies by B lymphocytes (64).

Oral lesions

Oral lesions are common and early manifestations, and some patients may have oral lesions without skin lesions. Initially vesiculobullous, the lesions readily rupture, new bulla developing as the older ones rupture and ulcerate (66), and thus erosions and ulcers are the main features and are seen mainly in the buccal mucosa, palate, and lips (50). Ulcers heal slowly, but scarring is rare (67). Gingival lesions usually include severe desquamative or erosive gingivitis, in which bullae have ruptured, to leave flaps of peeling tissue with red erosions or deep ulcerative craters mainly on the attached gingivae (68).

Diagnosis

In addition to a full history and examination, biopsy examination and appropriate histopathological and immunological investigations are frequently indicated. Biopsy of perilesional tissue, with histological and immunostaining examination, are essential to the diagnosis. Assay of serum antibody titers by indirect immunofluorescence (IIF) may also help guide prognostication and therapy. A recent critical evaluation of two enzyme-linked immunosorbent assays (ELISAs) for the detection of antibodies to Dsg1 and 3 comparing two substrates, normal human skin (HS) and monkey esophagus (MO), showed that with PV serum the sensitivity of IIF was 83% on HS and 90% on MO and that this combination of substrates should not only increase the sensitivity of detecting pemphigus antibodies but would aid in the differentiation of PV from PF (44). This strongly suggests that both substrates should be used in the diagnosis of PV, since patients with predominantly oral disease may only have Dsg3 antibodies, which are not always detectable with HS.

Therapy

Current treatment is largely based on systemic immunosuppression with systemic corticosteroids, using azathioprine, dapsone, methotrexate, cyclophosphamide, gold, and cyclosporin as adjuvants or alternatives. The recognition that the severity of the disease is related to the proportion of Dsg3 and Dsg1 antibodies (45) and to the titer of each (47) suggests that sequential assays to monitor the specificity and titer of antibodies, along with the clinical features, may be useful in determining the degree of immunosuppression needed. Localized oral lesions, with low-titer antibodies, may sometimes be treated with topical corticosteroids alone. Newer therapies such as mycophenolate mofetil or intravenous immunoglobulin therapy may prove in the longer term to offer significant advantages over the systemic corticosteroids.

Meanwhile, emerging therapies include the use of proteinase inhibitors (69), the development of chimeric molecules for specific recognition and elimination of the autoimmune B cells (70), and suggestions for targeting Dsg3-specific T cells to eventually modulate the T-cell-dependent production of pathogenic autoantibodies in PV (64). The anti-acantholytic activity of cholinergic agonists suggests a further novel avenue for the development of a non-hormonal treatment for PV (71).

Discussion and conclusions

The advent of new and improved technology has enabled much more detailed analysis of the structure and function of epithelium and of the BMZ. Analysis of autoantibody responses in patients with MMP has shown that distinct subgroups exist with either specific antigens or specific antibodies. The picture remains complex, but further studies should enable elucidation of the pathogenic role of the autoantibody–antigen complex and in the long term enable specific modulating immunotherapy.

New techniques in the diagnosis of pemphigus include specific ELISAs with recombinant major pemphigus antigens, Dsg1 and Dsg3. The Dsg3 ELISA is negative in PF and positive in PV, thus making it possible to discriminate between the two disease types. Dsg1 ELISAs are positive in both PF and PV. It is now clear that Dsg1 antibodies are detectable in 50% of PV and indicate a more severe phenotype. Furthermore, recent studies using these techniques have shown that oral pemphigus is associated with Dsg3 antibodies in the absence of skin involvement, whereas both antibodies are associated in patients with skin involvement. This may be useful in monitoring the disease and in prognostication.

Immunofluorescence is used more routinely, but, since mucosal tissues express greater levels of Dsg3 than skin, and, conversely, skin expresses more Dsg1 than mucosa, both substrates are necessary to improve the sensitivity of IIF in the diagnosis of pemphigus (44). Both the specificity and the antibody titer appear to have direct relationships with the disease severity, and this suggests that the tests should be carried out on a more routine basis and that a combination of clinical score and antibody titer provides valuable prognostic data. There is hope that earlier therapeutic intervention will both reduce complications and affect earlier remission.

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