Virchows Arch (1998) 432:487–504

REVIEW ARTICLE

Roland Moll · Ingrid Moll

Epidermal adhesion molecules and basement membrane components as target structures of autoimmunity

Received: 12 September1997 / Accepted: 27 October 1997

Abstract Intraepidermal and dermal-epidermal cohesion are of paramount importance for the integrity of the skin. Some constituent molecules of keratinocyte adhesion complexes and basement membrane-associated structures are the targets of antibody-mediated autoimmune reactions that give rise to various (muco-)cutaneous blistering diseases. The current state of our knowledge about these molecules – along with the main clinical, histological, and immunohistochemical features of the corresponding autoimmune diseases and their pathogenetic mechanisms – comprise the subjects surveyed in this review. Among the desmosomal cadherins (desmogleins and desmocollins) that mediate epidermal cell–cell adhesion, it has been demonstrated that desmoglein 1 and desmoglein 3 are the autoantigens of pemphigus foliaceus and pemphigus vulgaris, respectively, both diseases that result in intraepidermal blistering. Further, desmocollin autoantibodies may be involved in IgA pemphigus. Paraneoplastic pemphigus is associated with autoantibodies directed against the desmosomal plaque protein, desmoplakin. Of the constituents of hemidesmosomes, the plaque protein, BP230 (BPAG1), and the collagen-like transmembrane protein, BP180 (BPAG2), are the autoantigens of bullous pemphigoid and pemphigoid gestationis, the manifestations of both of which include subepidermal blistering. Several diseases arise from autoimmune reactions against certain proteins associated with the basement membrane located beneath hemidesmosomes, for example laminin 5 (cicatricial pemphigoid), ladinin (LAD-1; linear IgA disease), uncein, and collagen VII (epidermolysis bullosa acquisita), the last of which is the constituent protein of the anchoring fibrils. Such recent advances in the elucidation of the molecular nature of autoantigens may serve as the basis for the development of novel molecule-based therapeutic strategies.

Key words Desmosomal cadherins · Pemphigus · Hemidesmosomes · Basement membrane · Bullous skin diseases

R. Moll (✉)
Institute of Pathology, Martin Luther University of Halle-Wittenberg, D-06097 Halle, Germany
Tel.: +49-345-557 1882, Fax: +49-345-557 1295

I. Moll
Department of Dermatology, Universitätskrankenhaus Eppendorf, University of Hamburg, D-20246 Hamburg, Germany

Introduction

In multicellular organisms, cells are able to attach to other cellular and noncellular structures. The establishment and maintenance of adhesion – both between two identical or two different cell types (homophilic and heterophilic cell–cell adhesion) and between a cell and the extracellular matrix (cell–matrix adhesion) – are of fundamental importance in embryonic development and tissue homeostasis. Furthermore, cellular adhesion is a factor of great significance in various fields of pathology, including inflammation, immunopathology, and, by no means least, tumour pathology. In the last of these, the adhesive properties of tumour cells and their loss or acquisition of the ability to adhere to various structures of the host organism contribute to multiple events during tumour development and progression. Recent research has been able to cast a good deal of light on the molecules that form complex intra- or extracellular networks involving various interactions, thereby mediating diverse adhesion processes [17, 30, 51, 133, 134].

Another clinically important field in which defective cell–cell and cell–matrix adhesion are of pivotal importance with respect to pathogenesis is that of bullous autoimmune diseases of the skin. In these diseases, various adhesion molecules of epidermal keratinocytes and of the basement membrane zone are targets of humoral autoimmunity, leading to the loss of adhesion of epidermal cells at various (ultra)structural levels and, consequently, the formation of bullae (for an early review by pioneer researchers in this field, see [20]; for a more recent review see [130]). In recent dermatological review articles, the main features of these diseases have been summa-

rized as well as the particular methods, including immunofluorescence microscopy, required for their accurate diagnosis [66, 76]. The present survey of published work focuses on recent advances concerning the cell and molecular biology of epidermal adhesion molecules and considers their relevance with respect to the classification, pathogenesis, and treatment of autoimmune bullous diseases. As will be shown, not only has the molecular characterization of relevant autoantibodies been useful for clinical medicine, but such autoantibodies have also, in turn, been most valuable for cell biology, particularly basic cutaneous biology. Indeed, the identification of certain structural molecules, such as the antigens of pemphigus vulgaris and of bullous pemphigoid, has only been made possible by the use of sera from those affected [2, 31, 87]. Molecular sequencing of several of these antigens has revealed surprising relationships to known protein families, while unique, novel proteins have been identified in other cases.

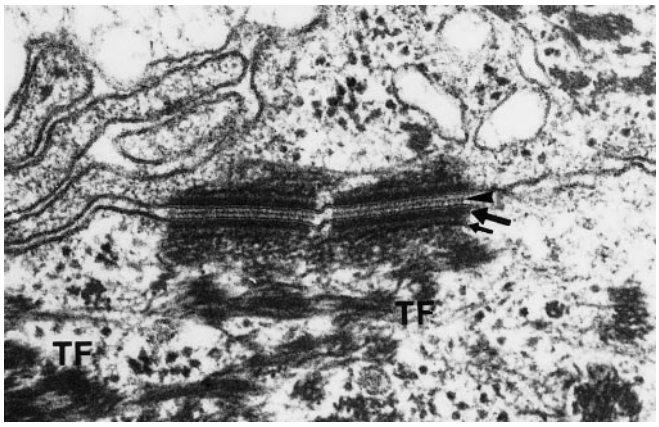

Fig. 1 Typical ultrastructure of desmosomes. Electron micrograph of two desmosomes connecting human epidermal stratum spinosum keratinocytes. Note the well-developed midline structure of the desmoglea (*arrowhead*), the conspicuous outer desmosomal plaque (*large arrow*) and the thinner inner plaque (*small arrow*) with the inserting tonofilaments (*TF*). ×55,000

Constituents and antigens of desmosomes

Among the intercellular adhering junctions of epithelial cells, desmosomes (maculae adhaerentes) are the most typical. In stratified squamous epithelia, these are particularly large and well formed, and their molecular composition is more complex than that of the desmosomes present in simple epithelia (for recent reviews, see [41, 42, 47, 48, 51, 86, 122, 130]). True desmosomes also occur in a few nonepithelial cell types, such as those of the myocardium and dendritic reticulum cells of lymphoid follicles.

The typical ultrastructure of desmosomes is shown in Fig. 1, and a simplified diagram of the structural and molecular composition of these junctions is presented in Fig. 2. The most prominent ultrastructural feature of desmosomes is the electron-dense plaque with a diameter of about 0.5 μm (Fig. 1). This can be differentiated into an outer (submembranous), very dense portion and an inner, less dense portion: bundles of intermediate filaments (in epithelial cells, cytokeratin filaments or tonofilaments) insert tangentially into the latter part. The desmosomal plaque mainly comprises the ubiquitous proteins, desmoplakin I (extending into the inner plaque) and plakoglobin (in the outer plaque), while particularly in stratified squamous epithelia, further, accessory proteins, such as desmoplakin II and plakophilin 1, may be also present ([122]; see also below). In addition, the two desmoplakin-related intermediate filament-associated proteins (IFAPs), plectin and IFAP 300, are also present in desmosomes (and in hemidesmosomes; see below) [51]. The intercellular adherence is assumed to be mediated by

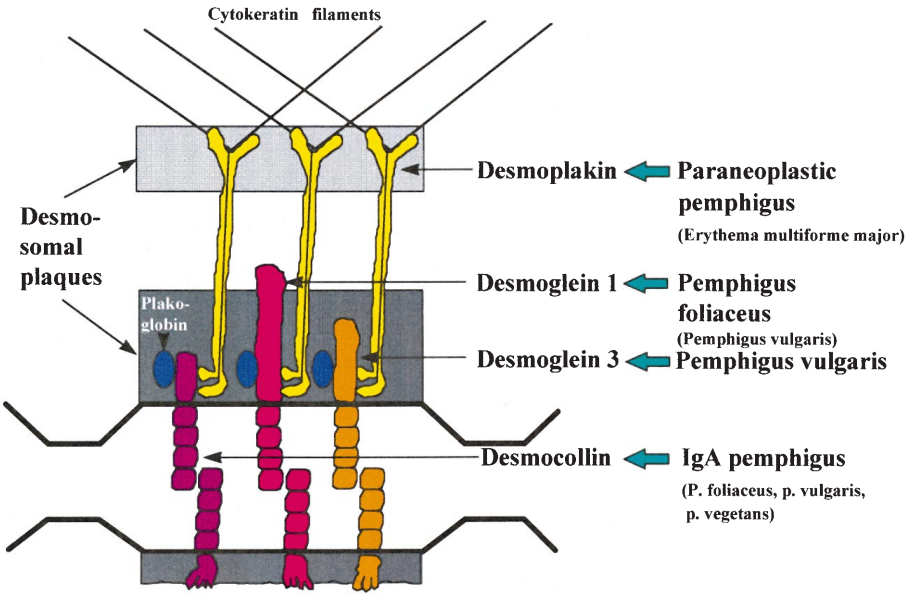

Fig. 2 Schematic illustration of the structural and molecular composition of the epidermal desmosome, together with the corresponding autoimmune diseases. The outer plaque is shown in *dark grey*, the inner plaque in *light grey*. Note that the extracellular domains of the desmosomal cadherins bridge the widened intercellular gap (only the outer part of the lower cell's desmosomal half is shown)

Table 1 Overview of autoantigenic cell-cell and cell-matrix adhesion proteins and basement membrane components and the corresponding autoimmune diseases

Structure	Autoantigen		Autoimmune diseases[a]	Blisters
	Name	Character		
Desmosomes	Desmoplakins	Cytoplasmic plaque proteins	Paraneoplastic pemphigus[b]; (erythema multiforme major)	Intraepidermal
	Desmoglein (Dsg) 1	Cadherin	Pemphigus foliaceus (including pemphigus erythematosus); pemphigus herpetiformis; (pemphigus vulgaris)	Intraepidermal
	Desmoglein (Dsg) 3	Cadherin	Pemphigus vulgaris (including pemphigus vegetans)	Intraepidermal
	Desmocollins (Dsc)	Cadherins	IgA pemphigus: Subcorneal pustular dermatosis (Dsc1), intraepidermal neutrophilic IgA dermatosis (Dsg3?); (pemphigus foliaceus); (pemphigus vulgaris, pemphigus vegetans)	Intraepidermal
Hemidesmosomes and associated basement membrane structures	BP230 (BPAG1)	Cytoplasmic plaque protein	Bullous pemphigoid; (pemphigoid gestationis)	Subepidermal
	BP180 (BPAG2)	Transmembrane protein (extracellular collagenous portion)	Bullous pemphigoid; pemphigoid gestationis; cicatricial pemphigoid	Subepidermal
	Laminin 5	Protein complex of anchoring filaments	Cicatricial pemphigoid (laminin-5 type)	Subepidermal
	Uncein	Protein complex of anchoring filaments	Cicatricial pemphigoid (uncein type; clinically epidermolysis bullosa acquisita-like)	Subepidermal
	Ladinin (LAD-1)	Component of anchoring filaments	Linear IgA disease, chronic bullous disease of childhood	Subepidermal
	Collagen VII	Component of anchoring fibrils	Epidermolysis bullosa acquisita; bullous systemic lupus erythematosus (type I)	Subepidermal

[a] Diseases in which autoantibodies against the respective autoantigen are of minor importance are indicated in brackets

[b] Autoantibodies against BP230 and a 190 kDa protein are also typically detected

transmembrane molecules, the desmosomal cadherins (desmogleins, desmocollins; for references, see below). The cytoplasmic portions of these molecules extend from the plasma membrane into the outer plaque. The extracellular portions of these molecules play a primary and immediate part in the mediation of intercellular adhesion occurring in a zipper-like manner; at the ultrastructural level these molecules have the appearance of a substance filling the distended intercellular gap (desmoglea) and exhibiting a dense midline structure recognizable in well-developed desmosomes. Thus, desmosomes anchor the intermediate-filament cytoskeleton to the plasma membrane, while simultaneously maintaining, via their role in cell–cell adhesion, the integrity and stability of multicellular epithelial tissue. As well as having these mechanical functions, desmosomes may also be involved in signal transduction pathways [30].

Several desmosomal proteins play important parts in certain bullous autoimmune diseases (Table 1; Fig. 2) and may serve as target antigens; as a result of the autoimmune processes, *intraepidermal blisters* develop.

Desmoplakins

The relatively large desmoplakin I (250 kDa) is an important, exclusively intracellular plaque protein present in all true desmosomes (Fig. 2). It is not found in most other plaque-bearing junctions, such as adherens junctions, and the only non-desmosomal junction in which desmoplakin (or a closely related protein) has been detected is the complexus adhaerens of the lymphatic endothelium and the retothelium of lymph nodes [120, 121]. Desmoplakin II is a smaller splice variant found in certain epithelia such as stratified squamous epithelia. Desmoplakins occur as homodimers, in which the central, intertwining α-helical domains of two molecules form a rod-like middle portion, from which the globular

N-terminal (head) and C-terminal (tail) domains protrude [86]. While the head is part of the electron-dense outer desmosomal plaque, the tail extends into the cell (inner plaque) and is prominently involved in the attachment of cytokeratin filaments [85]. Desmoplakin I is a ubiquitous component of all true desmosomes and is also detectable, as a marker, in malignant tumours derived from desmosome-bearing cells; along with carcinomas, such tumours include meningiomas and granulosa cell tumours [100].

Autoantibodies directed against desmoplakins I and II are typical features of the recently recognized *paraneoplastic pemphigus* [11, 57, 66, 109], a severe blister- and erosion-forming disease of the skin and mucous membranes, mainly occurring in patients suffering from malignant lymphomas (including chronic lymphatic leukemia) and thymomas.

Histologically, a prominent feature of this disease is epidermal basal-cell vacuolization, with resultant formation of suprabasal acantholytic blisters; further distinctive features are keratinocyte necroses and disseminated dyskeratoses, as well as the presence of a subepidermal inflammatory infiltrate.

It has been shown that when transferred to animals, the immunoglobulins of patients suffering from this disease exert pathogenic effects [11]; however, the precise mechanisms involved are unclear, since antibodies directed against intracytoplasmic desmoplakins cannot enter intact cells. Nonetheless, such anti-desmoplakin autoantibodies are of significance at least with respect to serodiagnosis, because in indirect immunofluorescence microscopy, they label various epithelia (including the urothelium) that are not stained by pemphigus autoantibodies (see below). Further autoantibodies that are present in paraneoplastic pemphigus and might be of pathogenic relevance are those directed against BP230 (see below) and against an as yet unidentified 190-kDa protein. Along with desmoplakins I and II, these antigens comprise the so-called paraneoplastic pemphigus complex (for a discussion on another 170-kDa transmembrane antigen with an extracellular domain, as well as atypical clinical and immunological varieties of paraneoplastic pemphigus and further references, see [28]).

Very recently, autoantibodies directed against desmoplakins I and II were found in a subset of patients with erythema multiforme major, some of whom had been exposed to nonsteroidal antirheumatic drugs and herpes simplex labialis [40]; the lesions seen in these patients had histopathological features reminiscent of those seen in cases of paraneoplastic pemphigus.

Plakoglobin and other desmosomal proteins of the *armadillo* family

Plakoglobin is a plaque protein (83 kDa) of the *armadillo* family, whose members are characterized by typically tandemly ordered repeating units and may also have signal transduction functions. This protein is also a component of nondesmosomal plaque-bearing junctions, such as actin-filament-associated adherens junctions, which typically also contain another *armadillo* protein, β-catenin (for a review, see [122]). In desmosomes (Fig. 2), plakoglobin is associated with the cytoplasmic portions of desmosomal cadherins (see below; [30, 41, 51]). Therefore, upon immunoprecipitation using pemphigus foliaceus and pemphigus vulgaris autoantibodies directed against certain desmosomal cadherins (see below), plakoglobin is precipitated along with the cadherins, although it is not itself an autoantigen [84, 130].

Two other *armadillo*-type proteins found in desmosomal plaques also occur in cell nuclei, a curious phenomenon whose significance is, as yet, unclear. These are plakophilin 1 (band-6 protein), whose presence in desmosomes is restricted to stratified and complex epithelia [62, 63, 71, 103, 122], and plakophilin 2, which has a more widespread desmosomal distribution [99]. Autoimmune phenomena related to these proteins have yet to be described.

Desmosomal cadherins

Desmosomal cadherins make an important contribution to the adhesive functions of desmosomes. These transmembrane glycoproteins belong to the supergene family of cadherins, which are calcium-dependent homophilic cell–cell adhesion molecules [49, 132–134], whose genes are clustered on chromosome 18q12. Interestingly, six desmosomal cadherins can be distinguished, these being differentially expressed according to the cell type and the stratified squamous epithelial layer. This group comprises three desmogleins (Dsg1, Dsg2, and Dsg3), which are characterized by relatively long cytoplasmic portions, and three shorter desmocollins (Dsc1, Dsc2, and Dsc3) [25, 41, 78, 86, 122, 130]. Of these, Dsg2 and Dsc2 appear to be expressed more or less throughout most desmosome-bearing tissues, while Dsg1, Dsg3, Dsc1, and Dsc3 are less widespread, being largely confined to stratified squamous epithelia [74, 101, 106, 107, 117, 118, 124, 137]. In comparison with the “classical” cadherins, E-, P-, and N-cadherin, the desmosomal cadherins differ principally in their cytoplasmic portions; however, they do exhibit relatively close homologies in their extracellular, calcium-ion-binding, repetitive domains, E1 (outermost, N-terminal) to E4, the outer domain(s) mediating homophilic binding to the corresponding portion of the partner molecule of a neighbouring cell (Fig. 2). The functional significance of certain desmosomal cadherins with respect to epidermal cell–cell adhesion is illustrated by their autoimmune pathology.

Desmoglein 1

Desmoglein 1 (Dsg1) is a desmosomal cadherin with a molecular weight of 160 kDa [79, 141], which has a rela-

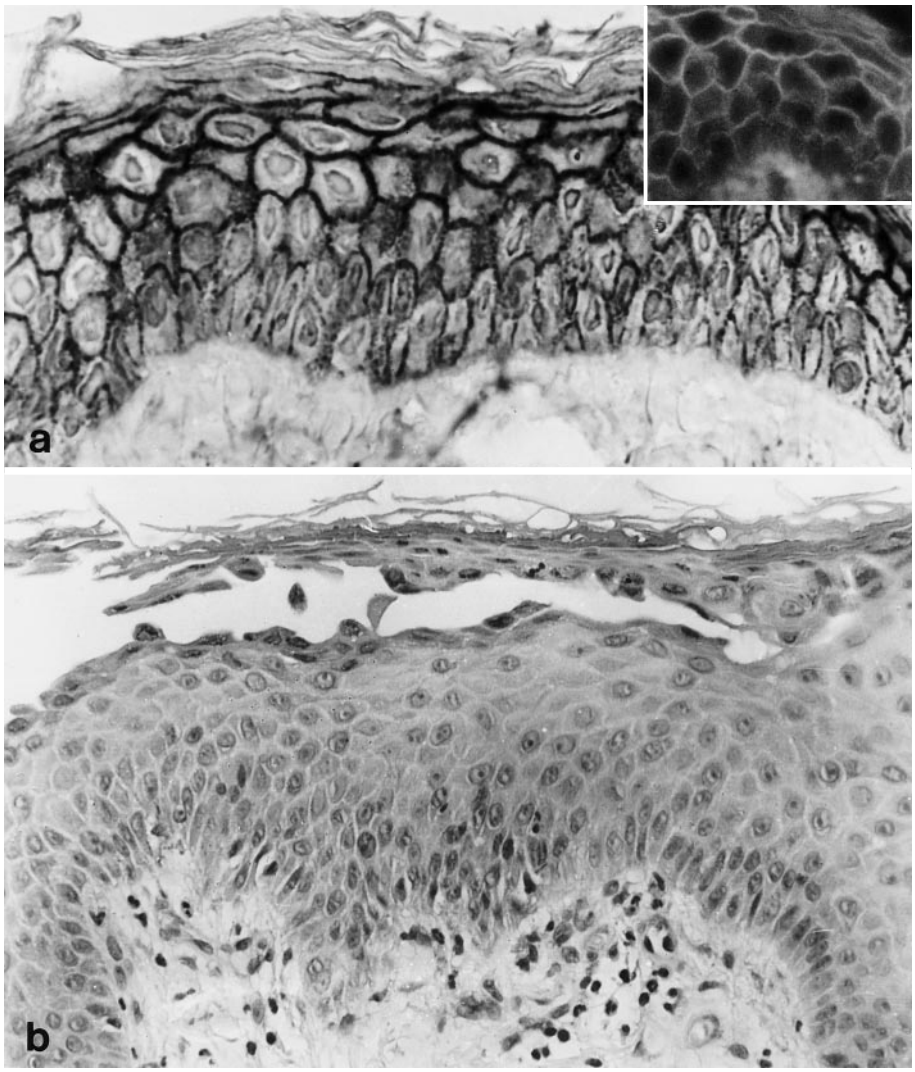

Fig. 3a, b Pemphigus foliaceus. **a** Indirect immunoperoxidase staining (applying 5 mM $CaCl_2$ [96]) of human epidermis detects circulating IgG4 autoantibodies with typical, suprabasally enhanced intercellular staining pattern (Brazilian endemic form). ×700. The *inset* illustrates a corresponding staining using the more common indirect immunofluorescence method. ×430. **b** Biopsy showing split formation at the level of the uppermost stratum spinosum and the stratum granulosum and a slight subepidermal inflammatory infiltrate. H&E, ×280. (Reprinted, with permission of Gustav Fischer Verlag Stuttgart, from [102])

tively long cytoplasmic tail extending into the desmosomal plaque (Fig. 2). Its expression is restricted to stratified squamous epithelia [75, 80, 117]. In the epidermis, Dsg1 is most prominent in the desmosomes of suprabasal layers, apparently attaining maximum expression in the upper epidermal layers [7, 12, 75, 125] while the immunostaining produced by autoantibodies against Dsg1 in basal epidermal cells is weaker but not insignificant (Fig. 3a; cf. [8]).

Dsg1 is the autoantigen of *pemphigus foliaceus* [84, 130]. The autoimmune nature and significance of autoantibodies in this disease, as well as in pemphigus vulgaris and bullous pemphigoid, was recognized in the pioneering work in this field by E.H. Beutner, R.E. Jordon, and T.P. Chorzelski [20]. Clinically, pemphigus foliaceus is characterized by highly superficial, flaccid skin blisters [111], whose extremely thin roofs rapidly rupture, giving rise to painful, crusted erosions with surrounding erythema. The lesions can affect the skin of the scalp, face, chest, and back, while mucosal sites are unaffected.

Besides the sporadic form, there is an endemic form of pemphigus foliaceus, fogo selvagem (wild fire; [32]), which is mainly confined to certain regions of Brazil and is thought to be transmitted by an insect (black fly) of the Simuliidae family [94]. In contrast to nonendemic pemphigus foliaceus, which affects mainly middle-aged and older persons, fogo selvagem mainly affects young persons (both Caucasians and Indians) living in rural areas; these patients share a common immunogenetic background displaying specific HLA alleles, which probably predispose them to the disease ([43]; see there for further references). The precise aetiology of pemphigus foliaceus, including that of fogo selvagem, is not completely understood. In the case of fogo selvagem, it is possible that black-fly saliva contains antigens that cross-react with Dsg1. In predisposed persons, these antigens would induce the production of pathogenic antibodies against Dsg 1, thus conforming with the mechanism of antigenic mimicry. Alternatively, the flies may transfer infectious agents, such as a presently unidentified virus. In some patients with pemphigus foliaceus and its variant, pemphigus erythematosus (see below), disease initiation or exacerbation has been observed after exposure to sunlight, while the induction of acantholysis

by artificial UV-B irradiation has been reported (for references, see [104]). Furthermore, certain drugs, particularly penicillamine, can induce autoantibody-mediated pemphigus-like disease [9].

When tested by application of indirect immunofluorescence or immunoperoxidase microscopy to human skin sections, circulating, pathogenic (see below) autoantibodies against Dsg1 (especially IgG4) produce a typical intercellular staining pattern of the epidermis, which may be suprabasally accentuated (Fig. 3a). On indirect immunoelectron microscopy, these antibodies have been found to bind to the extracellular part (desmoglea) of epidermal desmosomes [15, 112, 125]. Molecular analyses have shown that most pemphigus foliaceus autoantibodies are directed against epitopes on the in vivo-accessible, extracellular domain of Dsg1 [1, 6, 38]; some of these epitopes are conformational and calcium dependent [7].

In pemphigus foliaceus patients, direct immunofluorescence microscopy reveals that the distribution of in vivo-bound autoantibodies in the epidermis has a typical intercellular pattern [20, 66]. Such binding results in the destruction of the desmosomes and, histologically, in the formation of acantholytic gaps at the level of either the uppermost stratum spinosum or the stratum granulosum of the epidermis (Fig. 3b). Because of the very thin blister roof, intact blisters are rarely visible in histological specimens. There is a superficial dermal inflammatory infiltrate, often containing eosinophils [90]. Electron and immunoelectron microscopic studies have shown that in preacantholytic keratinocytes desmosomes become internalized ([131] and references cited therein) and possibly transformed into particular curvicircular bodies; it has been suggested that this may be the initial step in acantholysis [131].

The actual pathogenicity of the pemphigus foliaceus autoantibodies is underlined by the correlation between the antibody titres and the clinical severity of the disease [20]. Recently, experimental evidence that definitely demonstrates the pathogenicity of the anti-Dsg1-autoantibodies of pemphigus foliaceus has been obtained. When the immunoglobulins of such patients are passively transferred to newborn mice they produce typical blisters on the mouse skin. By means of absorption on the recombinantly constructed extracellular domain of Dsg1, the autoantibodies can be completely removed from the patient sera, and their experimental pathogenicity is entirely lost [6]. This opens up novel therapeutic perspectives with respect to antigen-specific plasmapheresis.

A variant form of pemphigus foliaceus exhibiting several clinical and immunological features of lupus erythematosus is called *pemphigus erythematosus* (Senear Usher syndrome), and again the target antigen is Dsg1 (see [66]). Another pemphigus variant, *pemphigus herpetiformis*, has features seen in dermatitis herpetiformis and typically exhibits eosinophilic spongiosis when subjected to histological analysis. This variant may be classified as pemphigus foliaceus (nonendemic form and endemic fogo selvagem) or, in some cases, as pemphigus vulgaris; the presence of autoantibodies directed against Dsg1 has been reported in this disease variant [115].

Desmoglein 2

The desmoglein isoform Dsg2, whose intracellular domain is larger than any other, is characterized by its occurrence in every desmosome-containing tissue studied up to now; thus, it is also present in the desmosomes of simple epithelia and of cardiomyocytes, for example [42, 117]. In stratified squamous epithelia, Dsg2 is confined to the basal cell layer, its level being particularly low in the epidermis, at which site it may be absent [101, 118, 119, 124]. Autoantibodies against Dsg2 have not yet been described; if they do exist, their presence would be expected to have severe consequences not only for internal simple epithelia (e.g., of the gastrointestinal tract) but also for the myocardium. There have been some reports concerning gastrointestinal involvement in patients with pemphigus ([123]; see there for further references), but such cases have not yet been subjected to analysis using autoantigens.

Desmoglein 3

Dsg3 (130 kDa), the pemphigus vulgaris antigen (PVA), is the (intracellularly) shortest of the three desmogleins (Fig. 2). Like Dsg1, it is essentially confined to stratified squamous epithelia [20, 117], but unlike Dsg1, Dsg3 is only present in the lowermost epidermal cell layers (basal and lower spinous layers) (Fig. 4a; [7, 8, 12, 72]; cf. also [125]). While, at the ultrastructural level, there is overall agreement concerning the general intercellular localization of this antigen at keratinocyte contacts, there have been discrepancies in published findings relating to its fine distribution. While some investigators have reported a more diffuse membrane distribution of PVA [19, 69], others have described a concentration of Dsg3 in desmosomes [72, 125]. In a very recent study involving the use of direct and indirect immunoelectron microscopy under native conditions, both the desmosomal desmoglea and large portions of the nondesmosomal keratinocyte membrane, containing adherens junctions, were found to be decorated by pemphigus vulgaris sera [16]. These findings suggest that Dsg3 occurs in both a desmosomal pool and, in smaller amounts, an interdesmosomal pool; however, it remains to be demonstrated whether Dsg3 is the only antigen in pemphigus vulgaris sera responsible for these results. That Dsg3 does indeed have an adhesive function has been demonstrated experimentally [5], although its capability in this respect is lower than that of classical cadherins.

The main target antigen of *pemphigus vulgaris*, the 130-kDa PVA (see [130]), has recently been identified as Dsg3 [2, 25]. However, beside anti-Dsg3 autoantibodies, some pemphigus vulgaris sera also contain autoantibodies directed against Dsg1 [6, 38, 58]. Clinically, com-

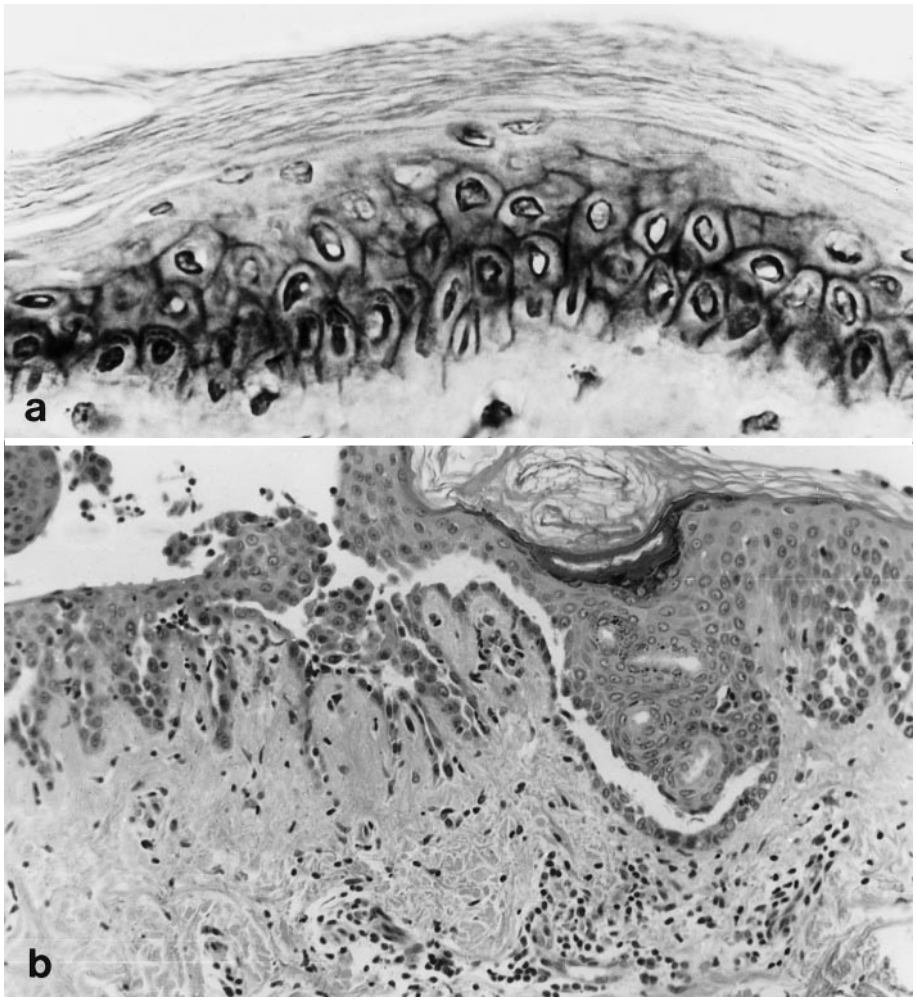

Fig. 4a, b Pemphigus vulgaris. **a** Intercellular decoration of human epidermis at the level of the stratum basale and lower stratum spinosum by IgG4 autoantibodies. Indirect immunoperoxidase staining, ×700. **b** Acantholytic split formation immediately above the basal cell layer, which extends onto an acrosyringium; slight inflammatory infiltrate in the upper dermis. H&E, ×180. (Reprinted, with permission of Gustav Fischer Verlag Stuttgart, from [102])

pared with pemphigus foliaceus, pemphigus vulgaris is a more severe disease, which before the corticosteroid era, always had a fatal outcome. Pemphigus vulgaris patients have thin-walled blisters and skin erosions (mainly face, trunk, pressure points, groin, and axillae) as well as the typical involvement of mucous membranes (particularly the oral mucosa), where slow-healing, painful erosions develop [111].

At present, our understanding of the aetiology of pemphigus vulgaris is incomplete. As discussed above with respect to pemphigus foliaceus, both genetic predisposition and exogenic influences are probably involved in the induction of the disease. The former is reflected by the prevalence of certain HLA genes in pemphigus vulgaris patients (for references, see [111]) and by the higher incidence of the disease in certain ethnic groups, such as Jews [111]. Possible exogenic influences include drugs, nutritional factors, viruses, emotional stress, and physical factors such as the UV radiation of sunlight ([104] and references cited therein). However, the mechanism(s) by which production of the pathogenic autoantibodies is initiated still remain(s) unclear.

Autoantibodies directed against Dsg3 are polyclonal. As revealed by immunoblotting and affinity purification using recombinant fusion proteins [3], a pathogenically relevant epitope is localized at the N-terminal region of the extracellular portion of the molecule (domains E1, E2), a region that is particularly important for the adhesive function of cadherins [105]. More frequently, though, conformation-dependent epitopes appear to be involved. It has been reported that a eukaryotically expressed (and therefore conformationally "correct") fusion protein containing the extracellular portion of Dsg3 was able to absorb the pathogenic autoantibodies out of nearly half of the pemphigus vulgaris sera studied [4]; the remaining sera required additional absorption using Dsg1 fusion protein (extracellular domain) to become entirely free of immunofluorescence-detectable autoantibodies [8]. The correct conformation of these artificial proteins depends on the presence of calcium ions, whose removal eliminates the binding of the autoantibodies [7]. In summary, all pemphigus vulgaris sera contain (pathogenic) autoantibodies directed against the extracellular domain of Dsg3, while the majority of these sera also contain (nonpathogenic) autoantibodies directed against the extracellular portion of Dsg1.

Being predominantly of the IgG4 subclass, circulating autoantibodies can be detected serologically by indirect

immunofluorescence microscopy, a procedure that is most effective when a calcium-ion-containing buffer is used [96]; indirect immunoperoxidase microscopy can be used to obtain comparable results (Fig. 4a). When examined by these methods, some pemphigus vulgaris sera produce intercellular staining of the lower epidermal cell layers (Fig. 4a) [8], which is in accordance with the known distribution of Dsg3; however, the majority of these sera bind throughout the entire living epidermis, a phenomenon that is due to the additional presence of autoantibodies directed against Dsg1 ([8] and references cited therein). Having bound to their target structures in vivo, i.e., to the epidermis and mucosal stratified squamous epithelia, these autoantibodies can be detected in biopsies, together with complement C3, by direct immunofluorescence microscopy, which reveals an intercellular staining pattern [20, 66]. Subsequently, there is observable widening of the intercellular space and dissolution of the "intercellular cement" both between basal and adjacent suprabasal cells, and laterally between the basal cells themselves, resulting in immediately suprabasal acantholysis [53]. The desmosomes are then destroyed; they may be internalized by endocytosis ([131] and literature cited therein). This pathomechanism is probably enhanced by the activation of complement and proteinases [3, 54]. However, the inactivation of Dsg3 via autoantibody binding alone would apparently be sufficient for the process of acantholysis, as shown by the fact that gene knock-out mice lacking Dsg3 exhibit, at certain sites, the kind of blisters typically seen in pemphigus vulgaris [81].

At the histological level (Fig. 4b), acantholytic blisters are apparent that are immediately suprabasal (with a split level considerably deeper than that seen in pemphigus foliaceus). In skin lesions, not only the epidermis but also hair follicles and sweat gland ducts become involved (Fig. 4b). Typically, basal cells remain attached to the basement membrane zone but their lateral connections become looser, resulting in the histological picture referred to as the "row of tombstones" pattern. Blister lumina often contain detached, rounded acantholytic keratinocytes, so-called pemphigus cells, while there is a mild superficial inflammatory infiltrate that often contains eosinophilic granulocytes.

When the immunoglobulins of patients with this disease are transferred to newborn mice, similar histological changes develop [3, 4, 10], thus conclusively demonstrating the pathogenic activity of these autoantibodies. Interestingly, anti-Dsg3 autoantibodies have been shown to be pathogenic, while those directed against Dsg1, which are frequently also detectable in pemphigus vulgaris, are not (this being in contrast to the anti-Dsg1 antibodies in pemphigus foliaceus) [4]. Even stronger evidence for the direct pathogenic significance of Dsg3 has recently been obtained by the induction, using recombinant Dsg3, of experimental rabbit antibodies producing pemphigus vulgaris like blisters in neonatal mice [98]. The ability shown in those recent studies [4, 98] to produce large quantities of pathogenically relevant, partial or complete Dsg3 protein has important implications for the development of specific immunotherapies for treating pemphigus vulgaris based on antigen-specific plasmapheresis (specific absorption using artificial antigens, such as recombinant fusion proteins bound to affinity columns). Such approaches would be highly desirable in view of the disadvantages of the present therapeutic schemes involving corticosteroids and nonspecific immunosuppressive agents, such as azathioprine [111].

A variant of pemphigus vulgaris that exhibits the same target antigen is *pemphigus vegetans*, which embraces the relatively benign but less common *Hallopeau type* and the more severe *Neumann type*, the main clinical characteristic of which is the occurrence of hypertrophic, serum-exuding granulations that later develop into verrucous plaques [66, 108, 111].

Desmocollins

The second subclass of desmosomal cadherins comprises the desmocollins (Fig. 2), which also occur in three subtypes (Dsc1, Dsc2, Dsc3). While, like the classical cadherins, their cytoplasmic portions are short, all three desmocollin isoforms occur in a longer (form "a") and a shorter (form "b") splice variant, which differ with respect to their cytoplasmic portions. Again, cell-type and stratum-specific differential expression is encountered: while Dsc2 is ubiquitous, Dsc3 is present in the desmosomes both of stratified squamous epithelia and of basal cells of pseudostratified epithelia, whereas Dsc1 is confined to the upper layers of keratinizing squamous epithelia, e.g., the epidermis [12, 74, 75, 106, 107, 137]. Thus, in desmosomes of the epidermis, while all three desmocollin subtypes are present their distribution is differential. The precise topographic and functional relationships between the individual desmogleins and desmocollins remain to be elucidated.

The clinical and pathogenetic significance of autoantibodies is less clear in the case of desmocollins than in that of desmogleins. Autoantibodies of the IgA type directed against desmocollins have been reported in patients suffering from *IgA pemphigus* [37, 60]. This disease entity – which has also been referred to as "intercellular IgA vesiculopustular dermatosis" [37] and "intraepidermal IgA pustulosis" [140] – is pustular rather than bullous in its clinical manifestation, in contrast to the other autoimmune dermatoses considered in the present review. It belongs to the group of neutrophilic dermatoses [140], and two subtypes can be distinguished: *subcorneal pustular dermatosis* (SPD), or Sneddon-Wilkinson disease, in which sterile pustules occur only in the upper epidermis [127], and *intraepidermal neutrophilic IgA dermatosis* (IEN), which involves pustule formation throughout the entire epidermis [37, 140]. Some cases are associated with monoclonal IgA gammopathies. In some (but not all) cases, low titres of circulating IgA autoantibodies demonstrating subcorneal or intercellular epidermal staining patterns can be detected by indirect

immunofluorescence. By definition, however, all cases are found to have intraepidermal IgA deposits – again in different patterns – when examined by direct immunofluorescence [140].

Interestingly, in a recent study, it was shown that SPD autoantibodies, binding to the upper epidermis only (as revealed by indirect immunofluorescence), recognized (bovine) Dsc1 when subjected to immunoblotting procedures; in contrast, IEN autoantibodies, which bind throughout the epidermis, reacted with (bovine) Dsc3 [60]. These binding patterns are identical to those revealed using experimental Dsc1 and Dsc3 antibodies, respectively [74, 107]. This suggests that, in these disease types, Dsc1 and Dsc3 may represent the actual target autoantigens, with the histological level of the pustular lesions corresponding very closely to the differential expression patterns of these two desmocollin isoforms. If this hypothesis is confirmed, the SPD and IEN subtypes of IgA pemphigus would have a similar relationship to each other in the desmocollin system to that of the pemphigus forms, pemphigus foliaceus and pemphigus vulgaris, in the desmoglein system. However, when tested on transgenically expressed, conformationally preserved *human* desmocollins, the reactivity of SPD autoantibodies has been convincingly demonstrated in a very recent study, while clarification of IEN autoantibodies with respect to their human antigenic targets has not yet been possible [61].

After in vivo autoantibody binding, IgA immune complexes may activate complement via the alternative pathway, thereby inducing neutrophil migration into their respective epidermal levels and subsequent tissue damage.

In addition to disease-typical desmoglein autoantibodies, autoantibodies directed against desmocollins have also been found in cases of Brazilian pemphigus foliaceus and, less frequently, in cases of nonendemic pemphigus foliaceus and pemphigus vulgaris [33, 58], as well as in two cases of Hallopeau-type pemphigus vegetans [55]; again, the pathogenetic significance of these findings remains unknown.

Hemidesmosomes

Hemidesmosomes are cell-matrix junctions that attach epithelial cells to the underlying basement membrane [21, 36, 51, 70, 82, 110, 130]. Together with specialized structures of the extracellular matrix (primarily anchoring filaments and anchoring fibrils), they form adhesion complexes that are intracellularly connected to the cytoskeletal network of cytokeratin filaments (tonofilaments). Hemidesmosomes are typically present in basal cells of the epidermis and of other stratified squamous epithelia, but they also occur in basal and myoepithelial cells of complex, pseudostratified, and glandular epithelia [110]. At the ultrastructural level (Fig. 5), although smaller, they have the appearance of halved desmosomes; however, molecular analyses – performed not least with the assistance of human autoantibodies – have shown that hemidesmosomes actually bear only a slight resemblance to desmosomes. In electron micrographs of these structures, the cytoplasmic, submembranous, electron-dense plaque is most conspicuous; in typical cases it is possible to distinguish an outer and inner plaque, with tonofilament bundles inserted into the inner plaque.

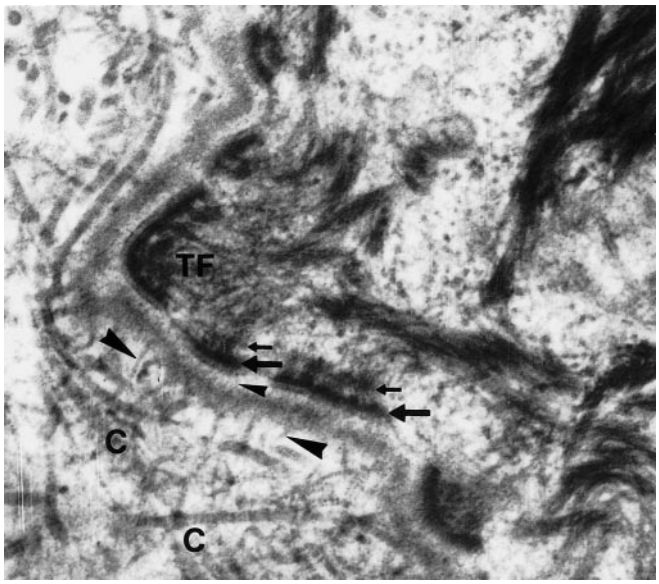

Fig. 5 Typical ultrastructure of hemidesmosomes, shown here in an electron micrograph of an epidermal basal cell (*right*; the dermis is to the *left*). Bundles of tonofilaments (*TF*) insert into the hemidesmosomal plaque (*small arrows* inner plaque, *large arrows* outer plaque). Fine anchoring filaments (*small arrowhead*) bridge the lamina lucida, while anchoring fibrils (*large arrowheads*) extend down from the lamina densa (*C* dermal collagen fibrils). ×54,000

Extracellularly, within the lamina lucida of the basement membrane, a subbasal dense plate is associated with the fine anchoring filaments that traverse the lamina lucida and insert into the lamina densa of the basement membrane. From the latter, root-like, collagenous anchoring fibrils extend into the subjacent dermal connective tissue (Fig. 5).

The molecular composition of hemidesmosomes is schematically depicted in Fig. 6. In brief (for details, see below), the hemidesmosome proper consists of several components [21, 47, 51, 70], including the cytoplasmic plaque protein BP230, the IFAPs plectin/HD1 (for the close relationship or identity of plectin and HD1, see [21, 44]) and IFAP300, and two transmembrane proteins, BP180 and $\alpha 6\beta 4$-integrin. BP230 and plectin and also the desmosomal plaque protein, desmoplakin, are related to each other, belonging to a single family of proteins [70, 93, 136]. The anchoring filaments appear to comprise several proteins. These include laminin 5, which has also been termed epiligrin, BM600, nicein, and kalinin [24, 113] and which may act as an extracellular ligand for $\alpha 6\beta 4$-integrin [26, 129]. Further anchoring filament components are laminin 6 (see [95]), uncein [65] and, possibly, the extracellular collagenous portion of

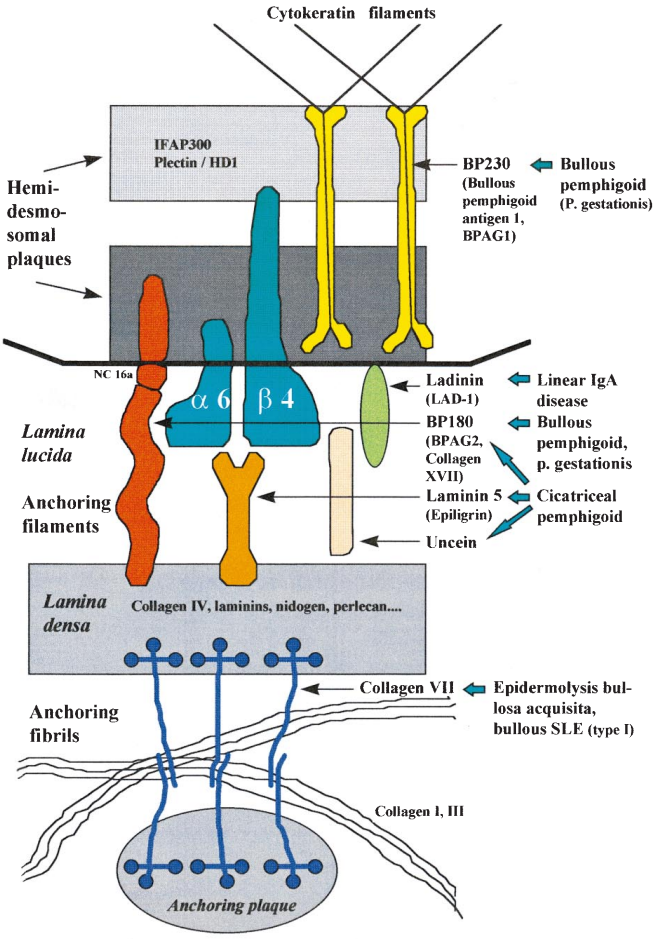

Fig. 6 Schematic illustration of the structural and molecular composition of the epidermal hemidesmosome, together with the corresponding autoimmune diseases. The outer plaque is shown in *dark grey*, the inner plaque in *light grey*. The lamina lucida is bridged by several different molecules, with transmembrane or entirely extracellular topography. Note the similarities between BP230 and desmoplakin (Fig. 2), both shown in *yellow*

BP180 [24, 70]. In contrast, the anchoring fibrils are entirely composed of collagen VII [23]. In the formation of hemidesmosomes, α6β4-integrin and plectin may form the core for hemidesmosome assembly [21], but laminin 5 also appears to be important for induction of the assembly process [14]. Autoimmune pathology indicates likely connections between many of these structural molecules and specific disease entities, all of which are characterized by *subepidermal blistering*, albeit at divergent ultrastructural split levels (Table 1, Fig. 6). In addition, defects of several hemidesmosome-associated proteins caused by specific gene mutations have recently been recognized as the molecular basis of such inherited bullous diseases as various forms of junctional epidermolysis bullosa, showing gene mutations for β4-integrin, BP180 or laminin 5 [21, 82].

Bullous-pemphigoid antigens

Two important structural components of hemidesmosomes have been identified and characterized with the help of autoantibodies obtained from patients with bullous pemphigoid: the bullous-pemphigoid antigens, BP230 and BP180 [130].

BP230 (BPAG1)

The major bullous-pemphigoid antigen, BP230 (also referred to as BPAG1), is a cytoplasmic (completely intracellular) 230-kDa protein of the hemidesmosomal plaque (Fig. 6). Its recently reported amino acid sequence [116] has revealed that it (together with plectin and IFAP300 [51, 93]) belongs to the same protein family as the desmosomal plaque protein, desmoplakin: indeed, these are the only known molecular relationships between desmosomes and hemidesmosomes [51, 130]. The genes for both BP230 and desmoplakin are localized on the short arm of chromosome 6 (BP230: chromosome 6p11). As is also true for desmoplakin, two BP230 molecules assemble into homodimers via their (α-helical central domains, which are flanked by N- and C-terminal globular domains, resulting in a structure resembling a double-dumbbell. Analogous to the situation for desmoplakin, the C-terminal domains are probably responsible for the binding of cytokeratin filaments. Very recent gene knock-out experiments have shown that BP230 is the main component of the inner hemidesmosomal plaque. In fact, this protein is of major importance for tonofilament binding, as is revealed by the fact that BP230-deficient mice lack both the inner plaque and inserting tonofilaments, resulting in highly exacerbated fragility of the basal cells in this submembranous zone; in contrast, the outer hemidesmosomal components (including the outer dense plaque) and their attachment to the basement membrane are unaffected in such mice [52].

BP180 (BPAG2; collagen XVII)

The minor bullous-pemphigoid antigen, BP180 [50, 70], this name being a reference to its size (180 kDa), is also called BPAG2. Its gene is localized on chromosome 10q24. BP180 is a unique transmembrane protein (Fig. 6) exhibiting an uncommon type-II orientation, i.e., with an extracellular C-terminus. While the intracellular (N-terminal) portion is very basic and is part of the (outer) hemidesmosomal plaque, the longer extracellular (C-terminal) portion contains 15 collagen-like domains. Thus, BP180 is very unusual in being a true cellular structural protein that exhibits features of a collagen molecule; because of these features, it is also referred to as collagen XVII. It has been postulated that the extracellular portions of three BP180 molecules combine into a collagen-typical triple helix. Obviously, this structure, which may alone or in part constitute the anchoring fila-

ments, has an important role in attachment of the hemidesmosomes to the extracellular matrix.

Autoantibodies directed against BP230 and BP180 are crucial in the pathogenesis of *bullous pemphigoid*. In this disease, which is usually encountered in elderly patients, generalized large blisters with tightly stretched roofs develop on partly erythematous skin, subsequently healing rapidly and leaving no scars [111]. In addition, blisters may occur in the oral mucosa. The spectrum of circulating autoantibodies is inhomogeneous, with the majority of sera (~70%) containing antibodies against BP230, ~55% of sera exhibiting antibodies against BP180, and both antibody species occurring together in ~30% of bullous-pemphigoid sera [56, 135]. The serum level of anti-BP180 autoantibodies has been found to be correlated with the prognosis for mortality, which appears to be worse than previously thought [18].

When indirect immunohistochemical methods are applied, all sera produce distinct linear staining of the epidermal basement membrane zone (Fig. 7a). When skin specimens that have been artificially split at the level of the lamina lucida by incubation in a 1 M NaCl solution ("split skin") are used as the test substrate, immunolabelling is usually observed on the epidermal side of the split, while the dermal side remains unstained (Fig. 7b). Direct immunofluorescence microscopy applied to the epidermis of patients with bullous pemphigoid reveals depositions of bound IgG and C3, these again exhibiting a linear staining pattern along the basement membrane zone [66, 76].

Although the antigenic epitopes present on BP230 appear to be localized on different portions of the molecule, they are preferentially situated in the C-terminal half [135]. In the case of BP180, an immediately extramembranous domain, NC16a (Fig. 6), represents an important antigenic epitope site [50]. This accords well with the observed binding of purified BP180 antibodies at the plasma membrane level of hemidesmosomes, as revealed by immunoelectron microscopy [67].

Subsequent pathophysiological events, which end with the detachment of basal epidermal cells from the basement membrane, can only be satisfactorily explained for BP180 autoantibodies binding on the extracellular side. Indeed, the transfer to newborn mice of experimentally produced rabbit antibodies directed against the NC16a epitope of BP180 has been reported to result in blisters [91]. In this experimental form of bullous pemphigoid, complement activation appears to have a pathophysiological role [92]. Hopkinson et al. [64] have speculated that BP180 autoantibodies might interfere with the interaction of BP180 and α6-integrin and thus perturb the structural integrity of the hemidesmosomes.

The pathogenetic significance of the more commonly encountered autoantibodies against intracytoplasmic BP230 – in intact cells in vivo, this should not be accessible to antibodies and, as outlined above in the context of knock-out experiments, it is not crucial for cell-matrix attachment proper [52] – currently remains unclear. In any case, bullous-pemphigoid autoantibodies bind in vivo to the basement membrane zone, thus appearing, on direct immunoelectron microscopy, as depositions of IgG in the lamina lucida in the regions of hemidesmosomes [90]. This results in the formation of a split at the level of the lamina lucida of the basement membrane zone and the destruction of hemidesmosomes [90]. These pathophysiological events are probably enhanced by second-

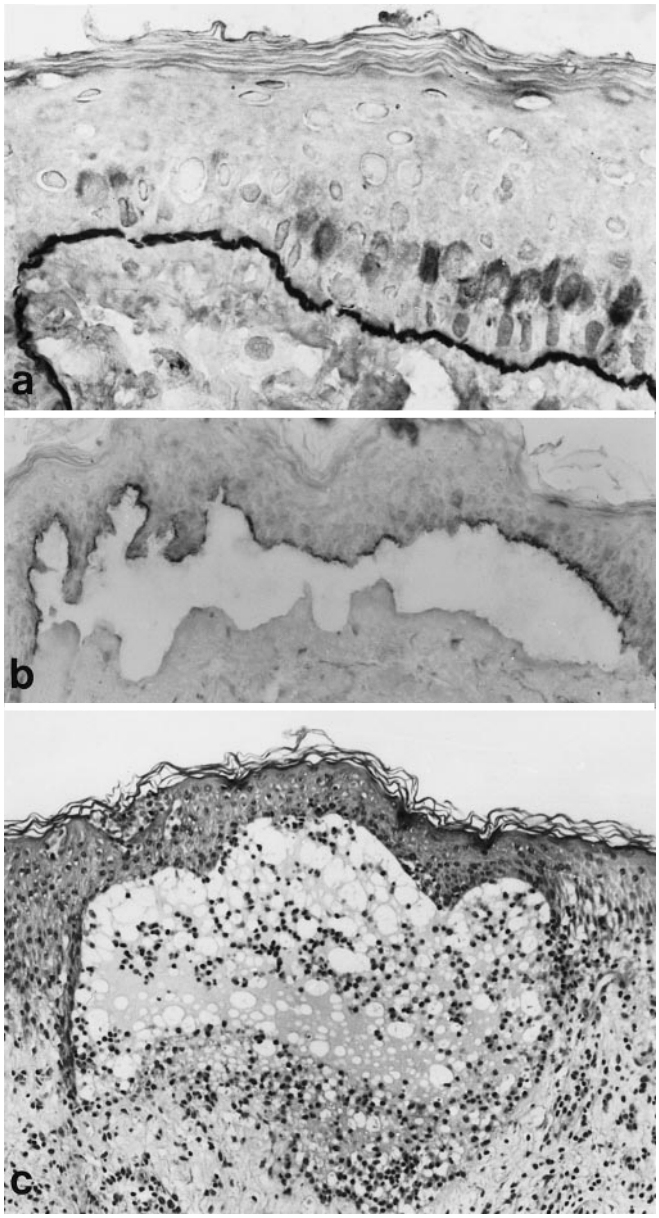

Fig. 7a–c Bullous pemphigoid. **a** Linear decoration of the epidermal basement membrane zone by IgG4 autoantibodies present in an autoimmune serum. Indirect immunoperoxidase staining, ×450. **b** In an artificial split skin specimen (1 M NaCl treatment), bullous pemphigoid antibodies decorate the epidermal side, indicating binding to hemidesmosomal components belonging to the basal epidermal cells proper (BP230, BP180; cf. Fig. 6). Indirect immunoperoxidase staining, ×190. **c** Typical subepidermal blister and mixed, eosinophil-rich inflammatory infiltrate penetrating into the blister lumen. H&E, ×110. (**a**, **c** Reprinted, with permission of Gustav Fischer Verlag Stuttgart, from [102])

ary mechanisms, such as the activation of complement, degranulation of mast cells, recruitment of leucocytes, and release of proteases [83, 92].

Histologically, bullous-pemphigoid lesions present as subepidermal blisters (Fig. 7c), with an infiltrate typically rich in eosinophilic leucocytes present in the adjacent dermis and blister lumen [90].

BP230 and, more commonly, BP180 are also target antigens in *pemphigoid (herpes) gestationis*, a rare dermatosis of pregnancy [50, 59, 66, 73, 76]. Starting in the periumbilical regions, such patients develop subepidermal blisters with an eosinophil-rich infiltrate. The autoantibodies involved are also referred to as "herpes gestationis factor"; one target epitope has been identified as being the NC16a domain of BP180 [50]. The placenta seems to play a part in the initiation of autoimmune events in this disease, together with abnormal expression of MHC class-II molecules [73].

The occurrence of autoantibodies against BP180 in a particular type of *cicatricial pemphigoid* is discussed below.

α6β4-Integrin

Integrins are heterodimeric transmembrane proteins of the cell surface that function as matrix receptors. The hemidesmosomal integrin complex, α6β4-integrin, is an important constituent of hemidesmosomes [47, 128, 129]. It is unique among the integrins owing to its cytoplasmic association with intermediate (cytokeratin) filaments (integrins are usually associated with actin microfilaments). The β4-chain has an unusually long cytoplasmic portion containing type-III fibronectin domains. Analogous to the intracellular portions of the desmogleins present in desmosomes, the cytoplasmic portion of β4-integrin is probably involved in the formation of the hemidesmosomal plaque. Laminin 5 (see below) is an important matrix ligand of α6β4-integrin. It appears likely that, as in the case of classical integrin-mediated cell–matrix junctions, ligand binding is coupled with signal transduction into the cell [21]. The high functional significance of α6β4-integrin for the integrity of hemidesmosomes [129] has been convincingly corroborated by the development of blisters in β4-integrin-deficient knock-out mice (see [21]); nevertheless, no corresponding autoimmune diseases have yet been described.

Laminin 5 (epiligrin, BM 600, nicein, kalinin)

Laminin 5 is a high-molecular weight (≥ 600 kDa) glycoprotein complex of epithelial basement membranes. It is identical to the autoantigen epiligrin and has also been referred to as BM 600, nicein, and kalinin [13, 24, 26, 34, 113]. Laminin 5 is a special variant of the classical, cross-shaped laminin 1 and consists of the three protein chains, α3, β3, and γ2 [24, 114]. Its β- and γ-chains are shorter than the corresponding laminin-1 chains (β1, γ1) and thus give rise to only rudimentary, short arms, resulting in a Y shape [14]. A small number of cysteine-rich epidermal growth factor (EGF) motifs are present in the N-terminal regions (short arms) of these laminin-5 chains [13]. Laminin 5 is localized in the hemidesmosomal lamina lucida, where it probably contributes to the formation of anchoring filaments (Fig. 6); as mentioned above, it is a ligand for hemidesmosomal α6β4-integrin (as well as for the α3β1-integrin of focal contacts) [26, 129]. The anchoring function of laminin 5 is evident not only from the autoimmune pathology described below, but also from the causal pathogenetic significance of mutations in the laminin-5 chain genes observable in certain forms of junctional epidermolysis bullosa (for a review, see [82]).

Autoantibodies directed against laminin 5 are relevant for a certain subtype of *cicatricial (mucosal) pemphigoid* [34, 35]. This is a chronic blister-forming disease whose main manifestations are in the oral mucosa, although other mucosal sites and the skin can also be involved [66, 76]. These mucosal blisters and erosions tend to result in scarring [111]. As can be shown by indirect immunofluorescence microscopy, autoantibodies directed against laminin 5 (α3-chain; [77]) bind to the dermal side of skin split with 1 M NaCl; indirect immunoelectron microscopy can detect these autoantibodies at the border between the lamina lucida and the lamina densa of the basement membrane, particularly in the region of hemidesmosomes at sites corresponding to the localization of anchoring filaments. When direct immunofluorescence microscopy is applied to split skin from biopsies of patients with cicatricial pemphigoid, IgG and C3 deposits are detectable at the same localizations [126].

The main histological features of this disease are subepithelial blisters and a chronic inflammatory infiltrate, with fibrosis and scarring often (in mucosal lesions consistently) developing subsequently [90].

In an experimental model, the administration of a monoclonal antibody against the α3-chain in skin fragments induced dermal–epidermal separation at the level of the lamina lucida, with bound antibody detectable on the dermal side of the antibody-induced split [113]. This suggests that antibody binding may directly displace preexisting protein–protein interactions, thus subsequently resulting in blistering. In cell culture, a monoclonal antibody against the globular (G) domain of the α3-chain of laminin 5 resulted in rapid detachment of hemidesmosomes from the underlying matrix [14]. Recently, a passive-transfer animal model in which laminin 5-antibodies directly elicited dermo-epidermal cleavage has been reported [89].

It should be noted, however, that cicatricial pemphigoid comprises a heterogeneous group of autoimmune diseases that can be distinguished from one another on the basis of split-skin immunofluorescence, immunoelectron microscopy, and immunoprecipitation [126]. A subtype more common than that mediated by laminin 5 is characterized by the presence of autoantibodies against BP180. As in the cases of bullous pemphigoid and pem-

phigoid gestationis, the juxtamembranous extracellular domain, NC16a, may be a target structure, although another target region of such autoantibodies is the C-terminal end region of BP180, which probably extends down to the lamina densa of the basement membrane [15].

Another lamina lucida antigen, protein p105, is the target antigen of an atypical blister-forming dermatosis that has recently been described for the first time [27].

Uncein

A further anchoring-filament component consisting of three polypeptide chains (165, 135, and 100 kDa), which is related to but distinct from laminin 5, is called uncein (Fig. 6; see [65]). Defects in uncein are known to be involved in the recessive junctional type of epidermolysis bullosa [39]. Recently, an acquired bullous dermatosis caused by autoantibodies against uncein has been described in one patient [65]. Electron microscopy and immunolocalizations revealed the lamina lucida to be the site of pathogenesis. This disease may be classified as a special form of cicatricial pemphigoid; clinically, the disease rather resembled epidermolysis bullosa acquisita (see below).

This and other forms of cicatricial pemphigoid (see above) illustrate that in the case of basement membrane zone molecules, autoimmune reactions (and mutations) involving different proteins and genes may result in a similar clinical phenotype.

Ladinin (LAD-1)

The molecular nature of the main autoantigen of the basement membrane-zone related, IgA-mediated autoimmune dermatosis, *linear IgA disease*, has very recently been elucidated. Another component of anchoring filaments (Fig. 6), it has a molecular weight of 97 kDa [95] and is synthesized and secreted by epidermal keratinocytes (in vivo, its molecular weight may be 120 kDa [95]). Using immunoelectron microscopy, this antigen has been found to be localized in the lamina lucida beneath hemidesmosomes [68]. Recent cloning of this molecule, termed LAD-1 [95] or ladinin [97], has revealed it to be a novel secretory protein (gene on chromosome 1q), which is very basic (pI 10.15) as a result of the enrichment of basic amino acids present in the N-terminal portion [97]. This feature may facilitate ionic binding to the basal plasma membrane of basal keratinocytes, thus contributing to the structural integrity of the basement membrane zone.

Ladinin appears to be the pathogenic autoantigen in most cases of linear IgA disease (linear IgA bullous dermatosis [68]). Beside the adult form, there is a paediatric variant called *chronic bullous disease of childhood*. The typical clinical picture in children comprises tightly stretched, small (1–3 mm) blisters at various skin sites, often grouped in rosettes around the edge of an annular lesion. Most patients also have mucosal involvement, very frequently with genital and often with ocular lesions, which subsequently cause scarring. In the adult form, the lesions are similar to those seen in children, or they may resemble acné excoriée [29].

While circulating IgA autoantibodies against ladinin are detected in such patients, the typical linear staining of the basement membrane zone seen by indirect immunofluorescence (in NaCl-split skin retained on the epidermal side) is not consistently observed in adults, and the autoantibody titres are usually low. However, with direct immunofluorescence, a consistent finding is the presence of a pathognomonic linear IgA band along the basement membrane zone. The bound antibody is predominantly of the IgA1 class [29].

As might be expected, histopathology [29] shows the presence of subepidermal blisters and a dermal infiltrate consisting predominantly of neutrophils and a few eosinophils. In some cases, the neutrophilic infiltrate may be concentrated in dermal papillae, resulting in papillary microabscesses resembling those seen in dermatitis herpetiformis, although the latter has a different IgA pattern (granular and papillary), as revealed by immunofluorescence [66].

The pathogenicity of IgA autoantibodies directed against ladinin is suggested by the finding that the incubation of unfixed human skin en bloc with an experimental monoclonal antibody against this protein induced dermal-epidermal separation in situ upon antibody binding to the anchoring filaments [95]. Incidentally, it should also be borne in mind that linear IgA disease is probably heterogeneous, as shown by the fact that some patients have been reported to exhibit immunolocalization patterns and autoantigens different from those described above (for references, see [29, 66, 68]).

Collagen VII

Collagen VII, which is mainly synthesized and secreted by keratinocytes, is the constituent molecule of the anchoring fibrils which, underneath the hemidesmosomes, attach the basement membrane to the underlying dermis [23]. At the ultrastructural level, these fibrils exhibit typical collagen banding [139]. Three chains of the α1(VII)-polypeptide (290 kDa; gene localized on chromosome 3p21) combine to form a triple helix typical of collagens, although this one is particularly flexible owing to the presence of several interruptions. At the N-terminal end, the triple helix is flanked by the large, noncollagenous, globular NC1 domain, whose complex composition includes multiple fibronectin type-III sequences. Two such triple-chain complexes associate at the opposite (C-terminal) ends to form a dimer resembling a dumbbell (Fig. 6). These dimers associate laterally to form anchoring fibrils [23, 139]. Since collagen VII interacts via its NC1 domains with other matrix proteins, such as collagen IV, the anchoring fibrils connect the collagen IV-containing lamina densa of the basement membrane with

the islet-like anchoring plaques in the subjacent dermis, which have a basement membrane-like composition and thus also contain collagen IV. These anchoring structures are interwoven with the dermal interstitial type-I and type-III collagen fibres, resulting in the adhesion of the basement membrane to the dermis.

Besides acquired autoimmune diseases, the pathology of type-VII collagen includes congenital mutations, that is to say the group of diseases that fall into the category of epidermolysis bullosa dystrophica [22, 23, 82, 138, 139].

The main autoimmune disease for which collagen VII acts as the target molecule is *epidermolysis bullosa acquisita*. Clinically, in skin that is initially free of inflammation, minor traumata induce blisters that leave pronounced scarring as well as milia and hyperpigmentation after healing [111]; the extensor surfaces of the extremities and the acra are the regions predominantly affected by this disease. Mucosal involvement is variable. The circulating IgG autoantibodies are directed against multiple epitopes (fibronectin type-III repeats) located in the noncollagenous NC-1 domain of collagen VII [46, 88]; indirect immunofluorescence microscopy reveals that these autoantibodies bind to the dermal side of NaCl-split skin. Applying direct immunofluorescence microscopy, IgG deposits appear as a broad band along the basement membrane zone [66, 76]. As revealed by immunoelectron microscopy, both the lamina densa and the dermal ends of the anchoring fibrils (anchoring plaques) are labelled, this corresponding to the localization of the NC-1 domains [76]. Although attempts at experimental passive transfer have not yet succeeded, these autoantibodies are considered to be pathogenic [88]. Histological examination reveals subepidermal blisters with a variable inflammatory infiltrate, which is usually absent in the classical noninflammatory phenotype but is, owing to the activation of complement, dense and rich in neutrophils in the inflammatory phenotype. In contrast to the situation in cases of bullous pemphigoid, eosinophils are sparse. During the course of disease, dermal scarring develops [76].

Another autoimmune disease that is associated with type-VII collagen autoantibodies is *bullous systemic lupus erythematosus (type I)*, which occurs predominantly in young black women. In addition to exhibiting all the features characteristic of systemic lupus erythematosus, this disease further includes extensive, nonscarring blistering resulting from an autoimmune reaction against collagen VII [45, 66]. The histological picture, which includes accumulations of neutrophils and papillary microabscesses along with subepidermal blistering, is reminiscent of that of dermatitis herpetiformis [45].

In both collagen-VII autoimmune diseases, genetic predisposition – an association with the HLA-DR-2 phenotype [45] – appears to play a part.

Conclusions

In the present review, classical examples of organ-specific autoantibody-mediated autoimmune diseases have been presented and discussed. In the last 10 years, there have been notable advances in the molecular-biological characterization and epitope mapping of the relevant autoantigens, while the significance of the pathogenicity of these autoantibodies has also been widely recognized. Nevertheless, many questions remain to be answered, for example with respect to the aetiological aspects of those diseases. Such questions include a possible genetic predisposition along with the fundamental problem of how the (frequently severe) autoimmune processes are initiated. Are infectious agents involved? What is the exact role of black-flies or viruses in endemic Brazilian pemphigus foliaceus? A frequently discussed aetiological hypothesis is that of molecular mimicry, i.e., the idea that extraneous molecules share antigenic epitopes identical with those of certain body's own proteins and thus initiate an autoantibody response. There are, however, good arguments in favour of stressing the importance of the autoantigen molecule itself in the initiation of the autoimmune response; for example, the frequent polyclonality of autoimmune sera suggests that at least a substantial portion of the authentic autoantigen molecule directs the immune response. But how, then, might endogenous molecules induce the formation of autoantibodies? One possibility is that aetiological factor(s) may slightly modify the relevant cell–cell and cell–matrix adhesion proteins or change their conformation, thereby releasing them from immune tolerance. Another open question concerns the pathogenic significance of autoantibodies directed against cytoplasmic antigens, such as desmoplakins and BPAG1, which are normally inaccessible to the immune system. Also, the role of T cells in supporting and modulating antibody-mediated autoimmune responses requires further clarification. Finally, there are still autoimmune dermatoses, such as dermatitis herpetiformis, whose target antigens have yet to be identified.

Nonetheless, the dramatic increase in available molecular-biological data concerning cell–cell and cell–matrix adhesion molecules has already had a considerable theoretical and practical impact. Thus, compared with the situation even a few years ago, we can now claim a much deeper understanding of the pathogenesis of many autoimmune diseases involving these molecules, as well as of related genodermatoses involving mutated or deficient adhesion molecules [82]. Practical, straightforward applications of such molecular data might take the form of molecularly defined therapeutic strategies, such as the development of antigen-specific plasmapheresis in the case of pemphigus. Such recent developments have arisen from the cooperative efforts of basic biological research and modern medicine.

Acknowledgements We would like to thank Prof. U. Bleyl (Institute of Pathology, Mannheim Faculty of Clinical Medicine, University of Heidelberg), Prof. W. Marsch (Clinic of Dermatology,

Martin Luther University of Halle-Wittenberg), and Dr. C. Bayerl (Clinic of Dermatology, Mannheim Medical School, University of Heidelberg) for providing histological specimens and various sera, and Prof. H. J. Holzhausen (Institute of Pathology, Martin Luther University of Halle-Wittenberg) and C. Grund (German Cancer Research Centre, Heidelberg) for providing electron micrographs. We also thank Dr. H. Bahn for performing immunofluorescence tests, A. Behringer for the preparation of the graphic work, A. Holzbach for technical assistance (all at the Institute of Pathology, Martin Luther University of Halle-Wittenberg), and A. Cowin (Heidelberg) for correcting the English.

References

1. Allen EM, Giudice GJ, Diaz LA (1993) Subclass reactivity of pemphigus foliaceus autoantibodies with recombinant human desmoglein. J Invest Dermatol 100:685–691
2. Amagai M, Klaus-Kovtun V, Stanley JR (1991) Autoantibodies against a novel epithelial cadherin in pemphigus vulgaris, a disease of cell adhesion. Cell 67:869–877
3. Amagai M, Karpati S, Prussick R, Klaus-Kovtun V, Stanley JR (1992) Autoantibodies against the amino-terminal cadherin-like binding domain of pemphigus vulgaris antigen are pathogenic. J Clin Invest 90:919–926
4. Amagai M, Hashimoto T, Shimizu N, Nishikawa T (1994) Absorption of pathogenic autoantibodies by the extracellular domain of pemphigus vulgaris antigen (Dsg3) produced by baculovirus. J Clin Invest 94:59–67
5. Amagai M, Karpati S, Klaus-Kovtun, Udey MC, Stanley JR (1994) Extracellular domain of pemphigus vulgaris antigen (desmoglein 3) mediate weak homophilic adhesion. J Invest Dermatol 102:402–408
6. Amagai M, Hashimoto T, Green KJ, Shimizu N, Nishikawa T (1995) Antigen-specific immunoadsorption of pathogenic autoantibodies in pemphigus foliaceus. J Invest Dermatol 104:895–901
7. Amagai M, Ishii K, Hashimoto T, Gamou S, Shimizu N, Nishikawa T (1995) Conformational epitopes of pemphigus antigens (Dsg1 and Dsg3) are calcium dependent and glycosylation independent. J Invest Dermatol 105:243–247
8. Amagai M, Koch PJ, Nishikawa T, Stanley JR (1996) Pemphigus vulgaris antigen (desmoglein 3) is localized in the lower epidermis, the site of blister formation in patients. J Invest Dermatol 106:351–355
9. Anhalt GJ (1993) Pemphigus: vulgaris, foliaceus, and paraneoplastic. In: Fine JD (ed) Bullous diseases. Igaku-Shoin, Tokyo, pp 52–74
10. Anhalt GJ, Labib RS, Voorhees JJ, Beals TF, Diaz LA (1982) Induction of pemphigus in neonatal mice by passive transfer of IgG from patients with the disease. N Engl J Med 306:1189–1196
11. Anhalt GJ, Kim SC, Stanley JR, Korman NJ, Jabs DA, Kory M, Izumi H, Ratrie H III, Mutasim D, Ariss-Abdo L, Labib RS (1990) Paraneoplastic pemphigus: an autoimmune mucocutaneous disease associated with neoplasia. N Engl J Med 323:1729–1735
12. Arnemann J, Sullivan KH, Magee AI, King IA, Buxton RS (1993) Stratification-related expression of isoforms of desmosomal cadherins in human epidermis. J Cell Sci 104: 741–750
13. Aumailley M, Krieg T (1996) Laminins: a family of diverse multifunctional molecules of basement membranes. J Invest Dermatol 106:209–214
14. Baker SE, Hopkinson SB, Fitchmun M, Andreason GL, Frasier F, Plopper G, Quaranta V, Jones JCR (1996) Laminin-5 and hemidesmosomes: role of the α3 chain subunit in hemidesmosome stability and assembly. J Cell Sci 109: 2509–2520
15. Balding SD, Prost C, Diaz LA, Bernard P, Bedane C, Aberdam D, Giudice GJ (1996) Cicatricial pemphigoid autoantibodies react with multiple sites on the BP180 extracellular domain. J Invest Dermatol 106:141–146
16. Bédane C, Prost C, Thomine E, Intrator L, Joly P, Caux F, Blecker M, Bernard P, Leboutet MJ, Tron F, Lauret P, Bonnetblanc JM, Dubertret L (1996) Binding of autoantibodies is not restricted to desmosomes in pemphigus vulgaris: comparison of 14 cases of pemphigus vulgaris and 10 cases of pemphigus foliaceus studied by Western immunoblot and immunoelectron microscopy. Arch Dermatol Res 288:343–352
17. Behrens J (1994) Cadherins as determinants of tissue morphology and suppressors of invasion. Acta Anat 149:165–169
18. Bernard P, Bédane C, Bonnetblanc J-M (1997) Anti-BP180 autoantibodies as a marker of poor prognosis in bullous pemphigoid: a cohort analysis of 94 elderly patients. Br J Dermatol 136:694–698
19. Beutner EH, Lever WF, Witebsky E, Jordon R, Chertock B (1965) Autoantibodies in pemphigus vulgaris: response to an intercellular substance of epidermis. JAMA 192:682–688
20. Beutner EH, Jordon RE, Chorzelski TP (1968) The immunopathology of pemphigus and bullous pemphigoid. J Invest Dermatol 51:63–80
21. Borradori L, Sonnenberg A (1996) Hemidesmosomes: roles in adhesion, signaling and human diseases. Curr Opin Cell Biol 8:647–656
22. Bruckner-Tuderman L (1991) Collagens of the dermo-epidermal junction: role in bullous disorders. Eur J Dermatol 1:89–100
23. Burgeson RE (1993) Type VII collagen, anchoring fibrils, and epidermolysis bullosa. J Invest Dermatol 101: 252–255
24. Burgeson RE, Chiquet M, Deutzmann R, Ekblom P, Engel J, Kleinman H, Martin GR, Meneguzzi G, Paulsson M, Timpl R, Tryggvason K, Yamada Y, Yurchenco PD (1994) A new nomenclature for laminins. Matrix Biol 14:209–211
25. Buxton RS, Cowin P, Franke WW, Garrod DR, Green KJ, King IA, Koch PJ, Magee AI, Rees DA, Stanley JR, Steinberg MS (1993) Nomenclature of the desmosomal cadherins. J Cell Biol 121:481–483
26. Carter WG, Ryan MC, Gahr PJ (1991) Epiligrin, a new cell adhesion ligand for integrin α3β1 in epithelial basement membranes. Cell 65:599–610
27. Chan LS, Wang X-S, Lapiere JC, Marinkovich MP, Jones JCR, Woodley DT (1995) A newly identified 105-kD lower lamina lucida autoantigen is an acidic protein distinct from the 105-kD γ2 chain of laminin-5. J Invest Dermatol 105:75–79
28. Chorzelski TP, Hashimoto T, Jablonska S (1996) Paraneoplastic pemphigus. Are autoantibodies pathogenic or an epiphenomenon? A reply. Eur J Dermatol 2:152–153
29. Collier PM, Wojnarowska F (1993) Linear IgA disease and chronic bullous disease of childhood. Eur J Dermatol 3:623–634
30. Cowin P, Burke B (1996) Cytoskeleton-membrane interactions. Curr Opin Cell Biol 8:56–65
31. Diaz LA, Calvanico NJ, Tomasi TB Jr, Jordon RE (1977) Bullous pemphigoid antigen: isolation from normal human skin. J Immunol 118:455–460
32. Diaz LA, Sampaio SAP, Rivitti EA, Martins CR, Cunha PR, Lombardi C, Almeida FA, Castro RM, Macca ML, Lavrado C, Filho GH, Borges P, Chaul A, Minelli L, Empinotti JC, Friedman H, Campbell I, Labib RS, Anhalt GJ (1989) Endemic pemphigus foliaceus (fogo selvagem). II. Current and historic epidemiologic studies. J Invest Dermatol 92:4–12
33. Dmochowski M, Hashimoto T, Chidgey MAJ, Yue KKM, Wilkinson RW, Nishikawa T, Garrod DR (1995) Demonstration of antibodies to bovine desmocollin isoforms in certain pemphigus sera. Br J Dermatol 133:519–525
34. Domloge-Hultsch N, Gammon WR, Briggaman RA, Gil SG, Carter WG, Yancey KB (1992) Epiligrin, the major human keratinocyte integrin ligand, is a target in both an acquired autoimmune and an inherited subepidermal blistering skin disease. J Clin Invest 90:1628–1633

35. Domloge-Hultsch N, Anhalt GJ, Gammon WR, Lazarova Z, Briggaman R, Welch M, Jabs DA, Huff C, Yancey KB (1994) Antiepiligrin cicatricial pemphigoid. A subepithelial bullous disorder. Arch Dermatol 130:1521–1529
36. Eady RAJ (1994) The hemidesmosome: a target in auto-immune bullous disease. Dermatology 189 [Suppl 1]:38–41
37. Ebihara T, Hashimoto T, Iwatsuki K, Takigawa M, Ando M, Ohkawara A, Nishikawa T (1991) Autoantigens for IgA anti-intercellular antibodies of intercellular IgA vesiculopustular dermatosis. J Invest Dermatol 97:742–745
38. Emery DJ, Diaz LA, Fairley AJ, Lopez A, Taylor AF, Giudice GJ (1995) Pemphigus foliaceus and pemphigus vulgaris autoantibodies react with the extracellular domain of desmoglein-1. J Invest Dermatol 104:323–328
39. Fine J-D, Horiguchi Y, Couchman JR (1989) 19-DEJ-1: a hemidesmosome anchoring filament complex-associated monoclonal antibody: definition of a new skin basement membrane antigenic defect in junctional and dystrophic epidermolysis bullosa. Arch Dermatol 125:520–523
40. Foedinger D, Sterniczky B, Elbe A, Anhalt G, Wolff K, Rappersberger K (1996) Autoantibodies against desmoplakin I and II define a subset of patients with erythema multiforme major. J Invest Dermatol 106:1012–1016
41. Franke WW, Troyanovsky SM, Koch PJ, Troyanovsky R, Fouquet B, Leube RE (1992) Desmosomal proteins: mediators of intercellular coupling and intermediate filament anchorage. Cold Spring Harbor Symp Quant Biol 57:643–652
42. Franke WW, Koch PJ, Schäfer S, Heid HW, Troyanovsky SM, Moll I, Moll R (1994) The desmosome and the syndesmos: cell junctions in normal development and in malignancy. In: Molecular and cellular basis for cell to cell interaction: Its significance in cancer (Proc. 24th Int Symp Princess Takamatsu Cancer Res Fund), Princeton Scientific Publishing, pp 14–27
43. Friedman H, Campbell I, Rocha-Alvarez R, Ferrari I, Coimbra CEA, Moraes JR, Flowers NM, Stastny P, Fernandez-Viña M, Olague-Alcala M, Diaz LA (1995) Endemic pemphigus foliaceus (fogo selvagem) in native Americans from Brazil. J Am Acad Dermatol 32:949–956
44. Gache Y, Chavanas S, Lacour JP, Wiche G, Owaribe K, Meneguzzi G, Ortonne JP (1996) Defective expression of plectin/HD1 in epidermolysis bullosa simplex with muscular dystrophy. J Clin Invest 97:2289–2298
45. Gammon WR, Briggaman RA (1993) Bullous SLE: a phenotypically distinctive but immunologically heterogeneous bullous disorder. J Invest Dermatol 100:28S-34S
46. Gammon WR, Murrell DF, Jenison MW, Padilla KM, Prisayanh PS, Jones DA, Briggaman RA, Hunt SW III (1993) Autoantibodies to type VII collagen recognize epitopes in a fibronectin-like region of the noncollagenous (NC1) domain. J Invest Dermatol 100:618–622
47. Garrod DR (1993) Desmosomes and hemidesmosomes. Curr Opin Cell Biol 5:30–40
48. Garrod D, Chidgey M, North A (1996) Desmosomes: differentiation, development, dynamics and disease. Curr Opin Cell Biol 8:670–678
49. Geiger B, Ayalon O (1992) Cadherins. Annu Rev Cell Biol 8:307–332
50. Giudice GJ, Emery DJ, Diaz LA (1992) Cloning and primary structural analysis of the bullous pemphigoid autoantigen BP180. J Invest Dermatol 99:243–250
51. Green KJ, Jones JCR (1996) Desmosomes and hemidesmosomes: structure and function of molecular components. FASEB J 10:871–881
52. Guo L, Degenstein L, Dowling J, Yu QC, Wollmann R, Perman B, Fuchs E (1995) Gene targeting of BPAG1: abnormalities in mechanical strength and in cell migration in stratified epithelia and neurologic degeneration. Cell 81:233–243
53. Hashimoto K, Lever WF (1967) An electron microscopic study on pemphigus vulgaris of the mouth and the skin with special reference to the intercellular cement. J Invest Dermatol 48:540–552
54. Hashimoto K, Shafran KM, Webber PS, Lazarus GS, Singer KH (1983) Anti-cell-surface pemphigus autoantibody stimulates plasminogen activator activity of human epidermal cells. J Exp Med 157:259–272
55. Hashimoto K, Hashimoto T, Higashiyama M, Nishikawa T, Garrod DR, Yoshikawa K (1994) Detection of anti-desmocollins I and II autoantibodies in two cases of Hallopeau type pemphigus vegetans by immunoblot analysis. J Dermatol Sci 7:100–106
56. Hashimoto T, Ebihara T, Ishiko A, Shimizu H, Bhogal BS, Black MM, Stanley JR, Nishikawa T (1993) Comparative study of bullous pemphigoid antigens among Japanese, British, and U.S. patients indicates similar antigen profiles with the 170-kD antigen present both in the basement membrane and on the keratinocyte cell membrane. J Invest Dermatol 100:385–389
57. Hashimoto T, Amagai M, Watanabe K, Chorzelski TP, Bhogal BS, Black MM, Stevens HP, Boorsma DM, Korman NJ, Gamou S, Shimizu N, Nishikawa T (1995) Characterization of paraneoplastic pemphigus autoantigens by immunoblot analysis. J Invest Dermatol 104:829–834
58. Hashimoto T, Amagai M, Watanabe K, Dmochowski M, Chidgey MAJ, Yue KKM, Garrod DR, Nishikawa T (1995) A case of pemphigus vulgaris showing reactivity with pemphigus antigens (Dsg1 and Dsg3) and desmocollins. J Invest Dermatol 104:541–544
59. Hashimoto T, Amagai M, Murakami H, Higashiyama M, Hashimoto K, Nishikawa T (1996) Specific detection of anti-cell surface antibodies in herpes gestationis sera. Exp Dermatol 5:96–101
60. Hashimoto T, Ebihara T, Dmochowski M, Kawamura K, Suzuki T, Tsurufuji S, Garrod DR, Nishikawa T (1996) IgA antikeratinocyte surface autoantibodies from two types of intercellular IgA vesiculopustular dermatosis recognize distinct isoforms of desmocollin. Arch Dermatol Res 288:447–452
61. Hashimoto T, Kiyokawa C, Mori O, Miyasato M, Chidgey MAJ, Garrod DR, Kobayashi Y, Komori K, Ishii K, Amagai M, Nishikawa T (1997) Human desmocollin 1 (Dsc1) is an autoantigen for the subcorneal pustular dermatosis type of IgA pemphigus. J Invest Dermatol 109:127–131
62. Hatzfeld M, Kristjansson GI, Plessmann U, Weber K (1994) Band 6 protein, a major constituent of desmosomes from stratified epithelia, is a novel member of the armadillo multigene family. J Cell Sci 107:2259–2270
63. Heid HW, Schmidt A, Zimbelmann R, Schäfer S, Winter-Simanowski S, Stumpp S, Keith M, Figge U, Schnölzer M, Franke WW (1994) Cell type-specific desmosomal plaque proteins of the plakoglobin family: plakophilin 1 (band 6 protein). Differentiation 58:113–131
64. Hopkinson SB, Baker SE, Jones JCR (1995) Molecular genetic studies of a human epidermal autoantigen (the 180-kD bullous pemphigoid antigen/BP180): identification of functionally important sequences within the BP180 molecule and evidence for an interaction betwen BP180 and α6 integrin. J Cell Biol 130:117–125
65. Horiguchi Y, Ueda M, Shimizu H, Tanaka T, Matsuyoshi N, Utani A, Ikai K, Nishikawa T, Imamura S (1996) An acquired bullous dermatosis due to an autoimmune reaction against uncein. Br J Dermatol 134:934–938
66. Huilgol SC, Bhogal BS, Black MM (1995) Immunofluorescence of the immunobullous disorders. Eur J Dermatol 5:186–195
67. Ishiko A, Shimizu H, Kikuchi A, Ebihara T, Hashimoto T, Nishikawa T (1993) Human autoantibodies against the 230-kD bullous pemphigoid antigen (BPAG1) bind only to the intracellular domain of the hemidesmosome, whereas those against the 180-kD bullous pemphigoid antigen (BPAG2) bind along the plasma membrane of the hemidesmosome in normal human and swine skin. J Clin Invest 91:1608–1615
68. Ishiko A, Shimizu H, Masunaga T, Hashimoto T, Dmochowski M, Wojnarowska F, Bhogal BS, Black MM, Nishikawa T (1996) 97-kDa linear IgA bullous dermatosis (LAD) antigen

localizes to the lamina lucida of the epidermal basement membrane. J Invest Dermatol 106:739–743
69. Jones JC, Yokoo KM, Goldman RD (1986) A cell surface desmosome-associated component: identification of a tissue-specific cell adhesion molecule. Proc Natl Acad Sci USA 83:7282–7286
70. Jones JCR, Asmuth J, Baker SE, Langhofer M, Roth SI, Hopkinson SB (1994) Hemidesmosomes: extracellular matrix/intermediate filament connectors. Exp Cell Res 213:1–11
71. Kapprell H-P, Owaribe K, Franke WW (1988) Identification of a basic protein of M_r 75,000 as an accessory desmosomal plaque protein in stratified and complex epithelia. J Cell Biol 106:1679–1691
72. Karpati S, Amagai M, Prussick R, Cehrs K, Stanley JR (1993) Pemphigus vulgaris antigen, a desmoglein type of cadherin, is localized within keratinocyte desmosomes. J Cell Biol 122:409–415
73. Kelly SE, Bhogal BS, Wojnarowska F, Whitehead P, Leigh IM, Black MM (1990) Western blot analysis of the antigen in pemphigoid gestationis. Br J Dermatol 122:445–449
74. King IA, Sullivan KH, Bennett R Jr, Buxton RS (1995) The desmocollins of human foreskin epidermis: identification and chromosomal assignment of a third gene and expression patterns of the three isoforms. J Invest Dermatol 105:314–321
75. King IA, O'Brien TJ, Buxton RS (1996) Expression of the "skin-type" desmosomal cadherin DSC1 is closely linked to the keratinization of epithelial tissues during mouse development. J Invest Dermatol 107:531–538
76. Kirtschig G, Wojnarowska F (1994) Autoimmune blistering disease: an up-date of diagnostic methods and investigations. Clin Exp Dermatol 19:97–112
77. Kirtschig G, Marinkovich MP, Burgeson RE, Yancey KB (1995) Antibasement membrane autoantibodies in patients with antiepiligrin cicatricial pemphigoid bind the α subunit of laminin 5. J Invest Dermatol 105:543–548
78. Koch PJ, Franke WW (1994) Desmosomal cadherins: another growing multigene family of adhesion molecules. Curr Opin Cell Biol 6:682–687
79. Koch PJ, Walsh MJ, Schmelz M, Goldschmidt MD, Zimbelmann R, Franke WW (1990) Identification of desmoglein, a constitutive desmosomal glycoprotein, as a member of the cadherin family of cell adhesion molecules. Eur J Cell Biol 53:1–12
80. Koch PJ, Goldschmidt MD, Zimbelmann R, Troyanovsky R, Franke WW (1992) Complexity and expression patterns of the desmosomal cadherins. Proc Natl Acad Sci USA 89:353–357
81. Koch PJ, Mahoney MG, Ishikawa H, Pulkkinen L, Uitto J, Shultz L, Murphy GF, Whitaker-Menezes D, Stanley JR (1997) Targeted disruption of the pemphigus vulgaris antigen (desmoglein 3) gene in mice causes loss of keratinocyte cell adhesion with a phenotype similar to pemphigus vulgaris. J Cell Biol 137:1091–1102
82. Korge BP, Krieg T (1996) The molecular basis for inherited diseases. J Mol Med 74:59–70
83. Korman NJ (1993) Bullous pemphigoid. Dermatol Clin 11:483–498
84. Korman NJ, Eyre RW, Klaus-Kovtun V, Stanley JR (1989) Demonstration of an adhering-junction molecule (plakoglobin) in the autoantigens of pemphigus foliaceus and pemphigus vulgaris. N Engl J Med 321:631–635
85. Kouklis PD, Hutton E, Fuchs E (1994) Making a connection: direct binding between keratin intermediate filaments and desmosomal proteins. J Cell Biol 127:1049–1060
86. Kowalczyk AP, Stappenbeck TS, Parry DAD, Palka HL, Virata MLA, Bornslaeger EA, Nilles LA, Green KJ (1994) Structure and function of desmosomal transmembrane core and plaque molecules. Biophys Chem 50:97–112
87. Labib RS, Anhalt GJ, Patel HP, Mutasim DF, Diaz LA (1986) Molecular heterogeneity of bullous pemphigoid antigens as detected by immunoblotting. J Immunol 136:1231–1235
88. Lapiere J-C, Woodley DT, Parente MG, Iwasaki T, Wynn KC, Christiano AM, Uitto J (1993) Epitope mapping of type VII collagen: identification of discrete peptide sequences recognized by sera from patients with acquired epidermolysis bullosa. J Clin Invest 92:1831–1839
89. Lazarova Z, Yee C, Cheng P, Yancey K (1996) Anti-laminin 5 IgG induces subepidermal blisters in adult murine skin as well as human skin grafted onto SCID mice. J Invest Dermatol 106:812
90. Lever WF, Schaumburg-Lever G (1990) Histopathology of the skin. Chapter 7: Noninfectious vesicular and bullous diseases. Lippincott, Philadelphia, pp 103–151
91. Liu Z, Diaz LA, Troy JL, Taylor AF, Emery DJ, Fairley JA, Giudice GJ (1993) A passive transfer model of the organ-specific autoimmune disease, bullous pemphigoid, using antibodies generated against the hemidesmosomal antigen, BP 180. J Clin Invest 92:2480–2488
92. Liu Z, Giudice GJ, Swartz SJ, Fairley JA, Till GO, Troy IL, Diaz LA (1995) The role of complement in experimental bullous pemphigoid. J Clin Invest 95:1539–1544
93. Liu CG, Maercker C, Castanon MJ, Hauptmann R, Wiche G (1996) Human plectin: organization of the gene, sequence analysis, and chromosome localization (8q24). Proc Natl Acad Sci USA 93:4278–4283
94. Lombardi C, Borges PC, Chaul A, Sampaio SAP, Rivitti EA, Friedman H, Martins CR, Sanches JA Jr, Cunha PR, Hoffmann RG, Diaz LA, Cooperative Group on Fogo Selvagem Research (1992) Environmental risk factors in endemic pemphigus foliaceus (Fogo selvagem). J Invest Dermatol 98:847–850
95. Marinkovich MP, Taylor TB, Keene DR, Burgeson RE, Zone JJ (1996) LAD-1, the linear IgA bullous dermatosis autoantigen, is a novel 120-kDa anchoring filament protein synthesized by epidermal cells. J Invest Dermatol 106:734–738
96. Matis WL, Anhalt GJ, Diaz LA, Rivitti EA, Martins CR, Berger RS (1987) Calcium enhances the sensitivity of immunofluorescence for pemphigus antibodies. J Invest Dermatol 89:302–304
97. Megahed M, Motoki K, McGrath J, La Forgia S, Uitto J (1997) Molecular cloning, structure, expression and chromosomal mapping of human and mouse ladinin genes. Arch Derm Res 289 [Suppl]:A 19
98. Memar OM, Rajaraman S, Thotakura R, Tyring SK, Fan J-L, Seetharamaiah GS, Lopez A, Jordon RE, Prabhakar BS (1996) Recombinant desmoglein 3 has the necessary epitopes to adsorb and induce blister-causing antibodies. J Invest Dermatol 106:261–268
99. Mertens C, Kuhn C, Franke WW (1996) Plakophilins 2a and 2b: constitutive proteins of dual location in the karyoplasm and the desmosomal plaque. J Cell Biol 135:1009–1025
100. Moll R, Cowin P, Kapprell HP, Franke WW (1986) Desmosomal proteins: new markers for identification and classification of tumors. Lab Invest 54:4–25
101. Moll R, Schäfer S, Koch PJ (1994) Molecular diversity of desmosomal cadherins and their potential as markers in the histodiagnosis of carcinomas. Verh Dtsch Ges Path 78:277–284
102. Moll R, Bahn H, Bayerl C, Moll I (1996) Zelluläre Adhäsionsmoleküle und Komponenten der extrazellulären Matrix als Zielstrukturen der Autoimmunität. Verh Dtsch Ges Pathol 80:67–79
103. Moll I, Kurzen H, Langbein L, Franke WW (1997) The distribution of the desmosomal protein, plakophilin 1, in human skin and skin tumors. J Invest Dermatol 108:139–146
104. Muramatsu T, Iida T, Ko T, Shirai T (1996) Pemphigus vulgaris exacerbated by exposure to sunlight. J Dermatol 23: 559–563
105. Nose A, Tsuji K, Takeichi M (1990) Localization of specificity determining sites in cadherin cell adhesion molecules. Cell 61:147–155
106. Nuber UA, Schäfer S, Schmidt A, Koch PJ, Franke WW (1995) The widespread human desmocollin Dsc2 and tissue-

specific patterns of synthesis of various desmocollin subtypes. Eur J Cell Biol 66:69–74
107. Nuber UA, Schäfer S, Stehr S, Rackwitz H-R, Franke WW (1996) Patterns of desmocollin synthesis in human epithelia: immunolocalization of desmocollins 1 and 3 in special epithelia and in cultured cells. Eur J Cell Biol 71:1–13
108. Ohata Y, Komiya H, Kawahara Y, Watanabe K, Nishikawa T, Hashimoto T (1996) A case of Neumann type pemphigus vegetans showing reactivity with the 130 kD pemphigus vulgaris antigen. Acta Derm Venereol 76:169–170
109. Oursler JR, Labib RS, Ariss-Abdo L, Burke T, O'Keefe J, Anhalt GJ (1992) Human autoantibodies against desmoplakins in paraneoplastic pemphigus. J Clin Invest 92:1775–1782
110. Owaribe K, Kartenbeck J, Stumpp S, Magin TM, Krieg T, Diaz LA, Franke WW (1990) The hemidesmosomal plaque. I. Characterization of a major constituent protein as a differentiation marker for certain forms of epithelia. Differentiation 45:207–220
111. Pye RJ (1992) Bullous eruptions. In: Champion RH, Burton JL, Ebling SJG (eds) Rook/Wilkinson/Ebling Textbook of Dermatology, 5th edn, vol 3. Blackwell, Oxford, pp 1623–1673
112. Rappersberger K, Roos N, Stanley JR (1992) Immunomorphologic and biochemical identification of the pemphigus foliaceous autoantigen within desmosomes. J Invest Dermatol 99:323–330
113. Rousselle P, Lunstrum GP, Keene DR, Burgeson RE (1991) Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments. J Cell Biol 114:567–576
114. Ryan MC, Tizard R, VanDevanter DR, Carter WG (1994) Cloning of the LamA3 gene encoding the α3 chain of the adhesive ligand epiligrin: expression in wound repair. J Biol Chem 269:22779–22787
115. Santi CG, Maruta CW, Aoki V, Sotto MN, Rivitti EA, Diaz LA, Cooperative Group on Fogo Selvagem Research (1996) Pemphigus herpetiformis is a rare clinical expression of nonendemic pemphigus foliaceus, fogo selvagem, and pemphigus vulgaris. J Am Acad Dermatol 34:40–46
116. Sawamura D, Li K, Chu M-L, Uitto J (1991) Human bullous pemphigoid antigen (BPAG1): amino acid sequences deduced from cloned cDNAs predict biologically important peptide segments and protein domains. J Biol Chem 266:17784–17790
117. Schäfer S, Koch PJ, Franke WW (1994) Identification of the ubiquitous human desmoglein, Dsg2, and the expression catalogue of the desmoglein subfamily of desmosomal cadherins. Exp Cell Res 211:391–399
118. Schäfer S, Stumpp S, Franke WW (1996) Immunological identification and characterization of the desmosomal cadherin Dsg2 in coupled and uncoupled epithelial cells and in human tissues. Differentation 60:99–108
119. Schäfer S, Nuber UA, Moll R, Franke WW (1997) Desmosomes and hemidesmosomes: differences and similarities in skin and mucosae. In: Pleyer U, Hartmann C, Sterry W (eds) Oculodermal diseases. Aeolus Press, Buren, The Netherlands, pp 75–94
120. Schmelz M, Franke WW (1993) Complexus adhaerentes, a new group ef desmoplakin-containing junctions in endothelial cells. I. The syndesmos connecting retothelial cells of lymph nodes. Eur J Cell Biol 61:274–289
121. Schmelz M, Moll R, Kuhn C, Franke WW (1994) Complexus adhaerentes, a new group of desmoplakin-containing junctions in endothelial cells. II. Different types of lymphatic vessels. Differentiation 57:97–117
122. Schmidt A, Heid HW, Schäfer S, Nuber UA, Zimbelmann R, Franke WW (1994) Desmosomes and cytoskeletal architecture in epithelial differentiation: cell type-specific plaque components and intermediate filament anchorage. Eur J Cell Biol 65:229–245
123. Schwermann M, Lechner W, Elsner C, Kirchner T (1988) Pemphigus vulgaris involving duodenum and colon. H+G Z Hautkrankh 63:101–104
124. Schwetlick I, Schäfer S, Franke WW, Moll R (1997) Desmoglein 2, a ubiquitous desmosomal cadherin, in carcinomas with compact and dissociated growth patterns. J Cancer Res Clin Oncol 123:Suppl 30
125. Shimizu H, Masunaga T, Ishiko A, Kikuchi A, Hashimoto T, Nishikawa T (1995) Pemphigus vulgaris and pemphigus foliaceus sera show an inversely graded binding pattern to extracellular regions of desmosomes in different layers of human epidermis. J Invest Dermatol 105:153–159
126. Shimizu H, Masunaga T, Ishiko A, Matsumura K, Hashimoto T, Nishikawa T, Domloge-Hultsch N, Lazarova Z, Yancey KB (1995) Autoantibodies from patients with cicatricial pemphigoid target different sites in epidermal basement membrane. J Invest Dermatol 104:370–373
127. Sneddon IB, Wilkinson DS (1979) Subcorneal pustular dermatosis. Br J Dermatol 100:61–68
128. Sonnenberg A, Calafat J, Janssen H, Daams H, Van der Raaij-Helmer LMH, Falcioni R, Kennel SJ, Aplin JD, Baker J, Loizidou M, Garrod D (1991) Integrin α6/β4 complex is located in hemidesmosomes, suggesting a major role in epidermal cell-basement membrane adhesion. J Cell Biol 113:907–917
129. Spinardi L, Einheber S, Cullen T, Milner TA, Giancotti FG (1995) A recombinant tail-less integrin β4 subunit disrupts hemidesmosomes, but does not suppress α6β4-mediated cell adhesion to laminins. J Cell Biol 129:473–487
130. Stanley JR (1993) Cell adhesion molecules as targets of autoantibodies in pemphigus and pemphigoid, bullous diseases due to defective epidermal cell adhesion. Adv Immunol 53:291–325
131. Tada J, Hashimoto K (1996) Curvicircular intracytoplasmic membranous structures in keratinocytes of pemphigus foliaceus. J Cutan Pathol 23:511–517
132. Takeichi M (1991) Cadherin cell adhesion receptors as a morphogenetic regulator. Science 251:1451–1455
133. Takeichi M (1993) Cadherins in cancer: implications for invasion and metastasis. Curr Opin Cell Biol 5:806–811
134. Takeichi M (1995) Morphogenetic roles of classic cadherins. Curr Opin Cell Biol 7:619–627
135. Tanaka M, Hashimoto T, Amagai M, Shimizu N, Ikeguchi N, Tsubata T, Hasegawa A, Miki K, Nishikawa T (1991) Characterization of bullous pemphigoid antibodies by use of recombinant bullous pemphigoid antigen proteins. J Invest Dermatol 97:725–728
136. Tang HY, Chaffotte AF, Thacher SM (1996) Structural analysis of the predicted coiled-coil rod domain of the cytoplasmic bullous pemphigoid antigen (BPAG1). Empirical localization of the N-terminal globular domain-rod boundary. J Biol Chem 271:9716–9722
137. Theis DG, Koch PJ, Franke WW (1993) Differential synthesis of type 1 and type 2 desmocollin mRNAs in human stratified epithelia. Int J Dev Biol 37:101–110
138. Uitto J, Christiano AM (1992) Molecular genetics of the cutaneous basement membrane zone: perspectives on epidermolysis bullosa and other blistering skin diseases. J Clin Invest 90:687–692
139. Uitto J, Chung-Honet LC, Christiano AM (1992) Molecular biology and pathology of type VII collagen. Exp Dermatol 1:2–11
140. Wallach D (1992) Intraepidermal IgA pustulosis. J Am Acad Dermatol 27:993–1000
141. Wheeler GN, Parker AE, Thomas CL, Ataliotis P, Poynter D, Arnemann J, Rutman AJ, Pidsley SC, Watt FM, Rees DA, Buxton RS, Magee AI (1991) Desmosomal glycoprotein DGI, a component of intercellular desmosome junctions, is related to the cadherin family of cell adhesion molecules. Proc Natl Acad Sci USA 88:4796–4800