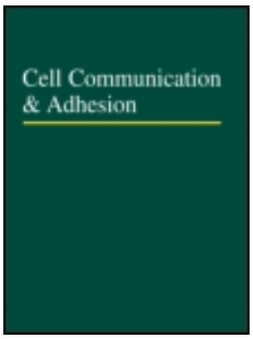

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REVIEW ARTICLE

Desmosomal adhesion and pemphigus vulgaris: the first half of the story

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Abstract

Pemphigus vulgaris (PV) is a paradigm of autoimmune disease affecting intercellular adhesion. The mechanisms that lead to cell–cell detachment (acantholysis) have crucial therapeutic implications and are currently undergoing major scrutiny. The first part of this review focuses on the classical view of the pathogenesis of PV, which is dominated by the cell adhesion molecules of the desmosome, namely desmogleins (Dsgs). Cloning of the DSG3 gene, generation DSG3 knock-out mice and isolation of monoclonal anti-Dsg3 IgG have aided to clarify the pathogenic mechanisms of PV, which are in part dependent on the fate of desmosomal molecules. These include perturbation of the desmosomal network at the transcriptional, translational, and interaction level, kinase activation, proteinase-mediated degradation, and hyper-adhesion. By the use of PV models, translational research has in turn helped shed light into the basic structure, function, and dynamics of assembly of desmosomal cadherins. The combined efforts of basic and applied research has resulted in tremendous advance into the understanding of epidermal adhesion and helped debunk old myths on the supposedly unique role of desmogleins in the mechanisms of cell–cell detachment in PV.

Keywords: cell adhesion, desmosome, pemphigus, Dsg3, keratinocytes

INTRODUCTION

A milestone in desmosome research was placed by the discovery of desmoglein 1 and 3 as autoimmune targets of pemphigus (Stanley et al., 1982; Amagai et al., 1991). This breakthrough drew considerable attention to the connection between desmosome and human disease (Cirillo et al., 2009a). Since, the study of autoimmune conditions caused by antibodies that are directed against desmosomal cadherins has contributed significantly to the understanding of the mechanisms of desmosomal adhesion.

Pemphigus (from greek *pemphix*, pustule) is a group chronic tissue-specific autoimmune blistering diseases targeting the skin and mucous membranes (Bystryn and Rudolph, 2005). The common lesion of all types of pemphigus is the disruption on cell–cell adhesion among keratinocytes with subsequent intraepithelial splitting, or acantholysis. Progress in the understanding of the nature of the autoantibodies to desmosomal components is illustrated in Figure 1.

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BLISTER FORMATION AND IMPAIRED FUNCTION OF DESMOGLEINS: AN APPARENTLY OBVIOUS CLINICAL-MOLECULAR CORRELATION

Pemphigus vulgaris (PV), the most common type of pemphigus, presents in middle-aged and genetically predisposed individuals with only oral lesions in one half to two thirds of patients, in contrast to pemphigus foliaceus (PF) in which mucous membrane involvement is usually absent. Oral blisters are fragile and rupture readily, leaving erosions which heal with difficulty (Cirillo, 2011). Mucosal dominant type of PV shows predominant oral erosions with limited skin involvement, which are no more than five or six erosions or blisters (Amagai, 1999). Subsequently, flaccid bullae may develop over several sites of the skin (trunk, scalp, flexures). Blistering is not always obvious, and often lesions consist of crusted erosions. In general, the muco-cutaneous type tends to be generalized and, therefore, more severe than the oral dominant type. Left untreated, the disease progresses with an almost always fatal outcome owing to uncontrolled fluid and protein loss or opportunistic infection (Baroni et al., 2007).

PV autoantibodies are detected bound to the surface of epidermis and circulating in patients' serum (Wolff and Schreiner, 1971; Patel et al., 1984; Shimizu et al., 2002). Serum titres seem to correlate

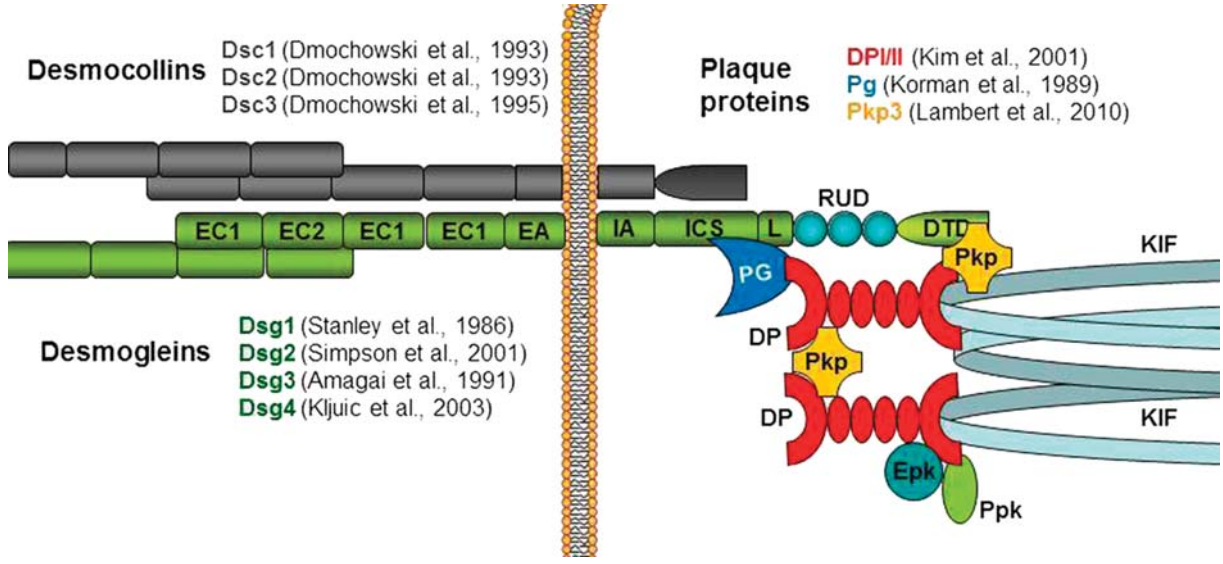

Figure 1. Schematic representation of the molecular constituents of the desmosome and their significance in pemphigus autoimmunity. The first reports describing desmosomal molecules as target antigens of pemphigus have been listed in the figure. For example, Stanley et al. reported in 1986 that Dsg1 was targeted by pemphigus autoimmunity, i.e. patients with pemphigus developed anti-Dsg1 IgG. Note that later studies have confirmed and further investigated the role of these antigens in the pathogenesis of pemphigus. Dsc, desmocollin; Dsg, desmoglein; DP, desmoplakin; Pg, plakoglobin; Pkp, plakophilin.

with the extent and the activity of PV as demonstrated by indirect immunofluorescence (IIF) titres as a measure of the pemphigus antibody levels (Mascarò et al., 1997; Harman et al., 2001).

The hallmark of histologic changes in pemphigus is the intra-epithelial cleft that results from cell–cell dyshesion (acantholysis) with formation of bullae containing isolated, round-shaped, acantholytic (or Tzanck) cells. The cleft is suprabasal in PV and pemphigus vegetans, subcorneal in the PF subtypes. A mild to moderate inflammatory infiltrate may be seen in the upper dermis (Baroni et al., 2007).

Work by Stanley's and Nishikawa's groups identified the epidermal antigen recognized by PV autoantibodies as a 130-kDa glycoprotein that was immunoprecipitated from keratinocyte extracts as part of the "PV complex", which also included the 85-kDa molecule plakoglobin (Stanley et al., 1982; Hashimoto et al., 1990; Amagai et al., 1991). The cDNA encoding the PV antigen has been then cloned and sequenced, identifying the antigen as desmoglein 3 (Dsg3) (Amagai et al., 1991). Studies to define the pathologic involvement of Dsg3 in acantholysis suggested that pemphigus autoantibodies induce lesions directly by interfering with the adhesive function of desmogleins through steric hindrance (Jones et al., 1986; Tsunoda et al., 2003; Nagasaka et al., 2004). In agreement with this theory, studies have demonstrated that the aminoacidic sequence held in the two N-terminal extracellular domains of Dsg3 is the immunodominant part of the molecule. It seems that PV-IgG recognizes the linear and conformational epitopes of Dsg3 formed by the N-terminal 161 amino acids, which contain sequences functionally critical for cadherin-mediated cell–cell adhesion (Amagai et al., 1992; Futei et al., 2000). Within this N-terminal region, most of the epitopes were mapped on residues 25–88 (Sekiguchi et al., 2001), 1–63, 89–161 (Tsunoda et al., 2003) and 50–79 (Bhol et al., 1995), just to name a few. It has been suggested that autoreactive T cells play a critical role in the induction and regulation of antibody production in pemphigus (Lin et al., 1997; Nishifuji et al., 2000), and that different subtypes of Dsg3-specific Th (Veldman et al., 2003) and Tr (Veldman et al., 2004a) cells are found in PV patients and healthy individuals. According to different authors, the peptides mainly recognized by auto-reactive T cells seem to be formed by residues 190–204, 206–220 (Wucherpfennig et al., 1995), 161–177 (Hertl et al., 1998) 96–112 and 250–266 (Veldman et al., 2004b), all located in the first two extracellular domains of Dsg3 (Dsg3EC1- 2). About one-half to two-thirds of PV sera also contain antibodies against Dsg1 and some of these react with Dsg4, although this finding is a result of the cross-reactivity of a small subset of anti-Dsg1 autoantibodies (Nagasaka et al., 2004). On the basis of the above findings, and putting as a corollary that Dsg1 and Dsg3 can reciprocally compensate their adhesive function, the site of blister formation in pemphigus was predicted and explained by Amagai (Amagai, 1999; Mahoney et al., 1999a). The few studies investigating the expression of desmogleins suggest that Dsg3 is expressed throughout the oral mucosa, especially in the upper two-thirds, whereas in the epidermis Dsg3 is reportedly expressed only in the basal and immediate suprabasal layer. Conversely, Dsg1 is found throughout the epidermis and oral mucosa, but more intensely in the subcorneal layer, and very weakly in the deep epidermis (Shirakata et al., 1998). Thus, PF anti-Dsg1 IgG cause blisters in the superficial layers of the epidermis but not

in the deep epidermis or mucosa, where the expression of Dsg3 compensates for the antibody-induced functional loss of Dsg1. Similarly, in mucosal PV, anti-Dsg3 IgG cause acantholysis in the deepest layer of mucous membranes, where Dsg1 expression is minimal. In mucocutaneous PV both anti-Dsg1 and anti-Dsg3 antibodies are required for pathogenicity (Amagai et al., 1999).

If this was correct, then why would muco-cutaneous (anti-Dsg1 and anti-Dsg3 positive) type PV develop deep, suprabasal acantholysis only, rather than diffuse intercellular detachment throughout the epidermis? One answer might be that cell–cell adhesion between the basal and immediate suprabasal layers might be weaker than the other parts of the epidermis because there are fewer desmosomes. In addition, the lower part of the epidermis might have better access for autoantibodies which penetrate from the dermis. This may explain why the splits become suprabasilar in mucocutaneous PV (Amagai, 1999).

A major flaw of this theory includes the undemonstrated assumption that Dsg1 and/or Dsg3 are essential for maintaining the adhesive properties of the epidermis. In fact, data show that inactivation of the Dsg3 gene or depletion of Dsg3 from keratinocytes within the epidermis of experimental animals or in cultured monolayers fails to induce gross skin blistering (Koch et al., 1997) or disrupt desmosomes (Aoyama and Kitajima, 1999; Chernyavsky et al., 2007), respectively. Furthermore, PERP-null mice and the conditional Dsc3null mutant mouse develop PV phenotype despite the presence of intact Dsg3 (Ihrie et al, 2005; Chen et al, 2008). In turn, patients with striate palmoplantar keratoderma featuring N-terminal deletion of Dsg1 do not develop acantholysis or skin blisters (Rickman et al, 1999).

The inconsistencies of the desmoglein compensation hypothesis have been detailed in a recent critical revision of the literature (Cirillo et al., 2012). Notwithstanding these obvious flaws, according to an old but still common view, the clinical phenotype of pemphigus is defined by the anti-desmoglein autoantibody profile (Amagai et al., 1999; Ding et al., 1997).

PEMPHIGUS VULGARIS AS A DISEASE OF THE DESMOSOME

It has been long assumed that autoantibodies against desmosomal cadherins are necessary and sufficient to induce acantholysis, making pemphigus one of the best characterized models to study the role of desmosome in cell adhesion and its dynamic rearrangement. The failure of desmosomal function in PV has been related to the following: 1. enhanced proteinase activity against molecules responsible for intercellular adhesion; 2. direct steric hindrance of desmoglein trans-interaction; 3. desmosome remodeling; 4. desmoglein signaling and down-regulation and/or altered function of desmosomal molecules; and 5. additional desmosomal antigens. The most important findings in the field are reported below:

Proteolytic cleavage of desmosomal cadherins

Early studies suggested that blister formation was mediated by the release of non-lysosomal proteases, such as plasminogen activator (PA), secondary to antibody binding (Schiltz et al., 1979). Although plasmin appeared to be the active enzyme in producing acantholysis (Dobrev et al., 1996), it is now accepted that none of these enzymes (i.e. plasmin and plasminogen activators) are necessary for pemphigus IgG-mediated acantholysis in mice (Mahoney et al., 1999b). However, the pathomechanisms occurring in PV patients' skin could be different from those triggered by highly concentrated PV IgG antibodies in the neonatal mouse model and, furthermore, proteases other than plasmin could be involved in acantholysis. The ability of MMP-9 to cleave Dsg3 during apoptosis (Cirillo et al., 2007a) and its limited proteolytic spectrum in comparison to plasmin made MMP-9 a major candidate for the pathogenesis of PV acantholysis.

Our group demonstrated for the first time that PV serum can induce sustained intracellular overexpression of proteinases such as MMP-9, followed by MMP-9 secretion. We have obtained evidence both in vitro, on keratinocyte monolayers and skin organ cultures, and in vivo, by establishing the neonatal mouse model of PV (Cirillo et al., 2007b). In light of our findings, we have proposed a novel explanation for PV pathogenesis (Cirillo et al., 2008a). Our hypothesis is based on the well-established selective proteolytic spectrum of a series of MMPs shown to be involved in the disruption of keratinocyte adhesion structures. For example, members of the ADAM (a disintegrin and metallopeptidase) family of MMP have been shown to mediate the proteolysis of cell adhesion molecules such as E-cadherin and Dsg1 (Steinhusen et al., 2001; Dusek et al., 2006), whereas Dsg3 is digested by enzymes belonging to the typical MMP family, namely MMP-9, during apoptosis (Weiske et al., 2001). These findings are intriguing, given the well-known ability of PV serum to induce apoptosis in keratinocytes both in vitro and in vivo (Puviani et al., 2003; Wang et al., 2004). Another class of proteases that appears to be central to both PV pathogenesis and cleavage of cell adhesion molecules are caspases. Specifically, caspase-3 inhibitors prevented PVIgG-induced acantholysis (Wang et al, 2004; Arredondo et al, 2005). Caspase 3 has been reported to cleave a series of cell adhesion molecules, including the desmosomal proteins Dsg1 (Dusek et al., 2006; Lanza and Cirillo, 2007), Dsg2 (Cirillo et al., 2008b), and Dsg3 (Weiske et al., 2001). More in general, caspases also target plaque proteins such as plakophilin-1 and desmoplakin-1 and -2 (Weiske et al., 2001), plectin, and periplakin (Aho, 2004; Kalinin et al., 2005). Taken together, these data strongly suggest that caspase activity is likely to play a fundamental role in weakening cell–cell adhesion during PV-associated apoptosis, now known as apoptolysis (Grando et al., 2009).

Direct steric hindrance of cell–cell adhesion

Early studies using in vitro models of PV suggested that cell–cell detachment was likely to occur as a consequence of the autoantibody binding to keratinocyte surface proteins and their internalization (Patel et al., 1984). Accordingly, Jones and co-workers hypothesized that acantholysis was generated from the direct interference of PV-IgG with the normal desmosomal functions (Jones et al., 1986). Years later, with the advent of modern technologies and the study of the expression pattern of the PV antigens Dsg1 and Dsg3 within the epidermis, together with the establishment of clinical, pathological and laboratory correlations of the disease, altogether led to the well-known "desmoglein compensation" theory (Amagai, 1999). Subsequent studies postulated that PV IgG could directly interfere with the adhesion function of desmogleins by steric hindrance with conformational epitopes of Dsg3 (Tsunoda et al., 2003; Nagasaka et al., 2004). Immunomapping of pemphigus immune globulins has indeed demonstrated that pathogenic antibodies bind the amino-terminal extracellular domain of Dsgs that is predicted to form the trans-adhesive interface between cells (Tsunoda et al., 2003; Sekiguchiet al., 2001; Li et al., 2003), however no dyscohesive effect is induced by IgG to linear epitopes of Dsg3 (Cirillo et al., 2009b). These findings partially served as a further validation of the compensation hypothesis, although it should be emphasized that the two concepts do not necessarily overlap. The main findings in support to the desmoglein compensation theory have been reported in detail by us in previous review articles (Lanza et al., 2006; Cirillo et al., 2012).

Even assuming that competitive binding of IgG to the adhesive epitopes of Dsg3 is sufficient to induce acantholysis, it is not yet clear whether PV IgG are able to bind desmosome-assembled Dsg3 or, rather, block its extracellular domain before Dsg3 engages contacts with apposed cells. With regard to this, it is interesting that detachment of keratinocytes from each other seems to occur first in the interdesmosomal areas, while desmosomes appear to separate only in the late acantholysis (Bystryn and Grando, 2006). It also remains to address whether IgG-Dsg3 interactions can cause desmosome splitting or prevent formation of new desmosomes, respectively. More recent findings support the view that PVIgG bound to unassembled desmosomal cadherins does not prevent desmosome generation; rather, it leads to internalization and possibly degradation of IgG-antigen complex (Aoyama et al., 2010).

The steric hindrance model was questioned by the report that PF IgG caused dissociation of keratinocytes without blocking Dsg1 homophilic trans-interaction (Waschke et al., 2005). It should be noted that the steric hindrance model does not imply a passive, non-energy dependent process, as studies have shown that keratinocytes incubated with PV IgG at 4°C do not demonstrate decreased intercellular adhesion in a dispase assay (Calkins et al., 2006).

Instead, this model likely ties into mechanisms of desmosome rearrangement, assembly and disassembly, and signaling, which will be reviewed in the next paragraphs.

Desmosome remodeling

It has been recently suggested that PV IgG may work through the depletion of Dsg3 from keratinocytes that follows its internalization and degradation, possibly depriving the cell from free Dsg3 not yet assembled into desmosomes (Yamamoto et al., 2007). It is not yet clear, however, whether Dsg3 depletion is pathogenetically relevant: depletion of Dsg3 *in vivo* is only modest or absent (Shu et al., 2007); with regard to *in vitro* studies, it is well known that trans-membrane molecules, including receptors, become internalized, degraded and/or recycled after binding to antibodies or ligands. Therefore, the depletion of Dsg3 may be a secondary event resulting from the production of anti-Dsg3 IgG and/or an epiphenomenon related to inside-out signaling caused by PV sera (Figure 2).

In this section, we will be focusing on the specific changes affecting both Dsg3 and desmosome formation in PV acantholysis.

Changes in Dsg3 turn-over, internalization and assembly into desmosomes

Reduction of Dsg3 levels from cell lysates has been reported after incubation of keratinocytes with PV sera, PV IgG, and anti-Dsg3 mAb. The binding of PV IgG to their membrane targets is thought to affect primarily the Triton X-100-soluble, free membrane pool of Dsg3, whereas the delayed depletion of Dsg3 from Triton X-100-insoluble pools would reflect the subsequent abrogation of Dsg3 incorporation into desmosomes (Aoyama and Kitajima, 1999; Aoyama et al., 1999). Study by Yasuo Kitajima demonstrated that different anti-Dsg3 monoclonal antibodies of mouse origin exerted cumulative or synergistic effects in depleting keratinocytes and desmosomes of Dsg3 (Yamamoto et al., 2007). In marked contrast, Waschke and colleagues reported that PV IgG increased Dsg3 in the triton-soluble fraction of cell lysates, indicating that the anchorage of Dsg3 to the cytoskeleton was reduced by pemphigus IgG (Waschke et al., 2006). They also demonstrated that Dsg3 levels were not substantially decreased in the cytoskeleton fractions after 24 h of incubation with PV IgG. In our opinion, these changes of Dsg3 levels in the triton soluble pool of proteins may be interpreted as either depletion of free membrane Dsg3 (if binding to non-desmosomal Dsg3 is predominant) or titration from the triton-insoluble to triton-soluble fraction (if binding to desmosomal Dsg3 with subsequent detachment from cytoskeleton is the pivotal event).

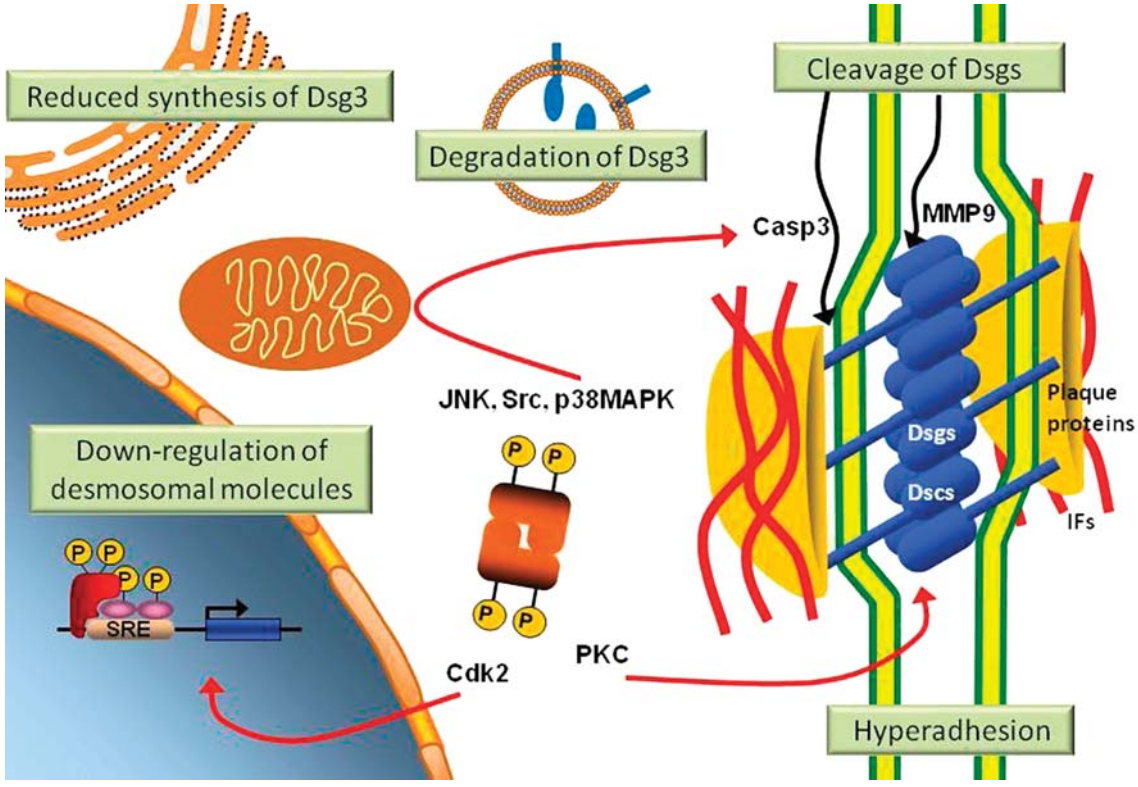

Figure 2. Intracellular pathways involved in the disruption of desmosomal adhesion in pemphigus. The acantholytic mechanisms target adhesion molecules as a result of both direct processing and indirect intracellular signaling. They include the internalization and degradation of Dsg3, apoptotic signals that lead to activation of caspases (e.g., Casp3) and metalloproteinases (e.g., MMP9), hyperadhesion, and transcriptional/translational down-regulation of desmosomal molecules. As shown in the picture, most of the changes are orchestrated by protein kinases. Dsgs, desmogleins; Dscs, desmocollins; IFs, intermediate filaments; Casp3, Caspase 3; MMP9, matrix metalloproteinase 9.

We have demonstrated for the first time that an effect of PV serum is the reduction of Dsg3 half-life in keratinocytes (Cirillo et al., 2006). This finding could be related to the ability of PV autoantibodies to interfere with the stability of non-desmosomal Dsg3 but not of properly assembled desmosomes. Furthermore, binding of PV IgG to Dsg3 perturbs its recruitment at sites of intercellular contact and subsequent assembly into desmosomes. Our evidence has suggested that PV IgG does not cause desmosomal split, as instead has been previously reported in a mouse model of PV (Shimizu et al., 2004). In support of our view that PV IgG does not directly split the desmosome, evidence by Kowalczyk's group has revealed that pharmacological inhibition of Dsg3 endocytosis prevents disruption of desmosomes and loss of adhesion in the presence of PV IgG. Their results also suggest that PV IgG-induced Dsg3 internalization is mediated through a clathrin and dynamin-independent pathway, and that Dsg3 endocytosis is tightly coupled to the pathogenic activity of PV IgG (Delva et al., 2008).

Processing of Dsg3 during acantholysis

Because abrogation of Dsg3 from keratinocytes reportedly parallels cell–cell detachment (Yamamoto et al., 2007; Aoyama and Kitajima, 1999), it is reasonable that Dsg3 processing and acantholysis are related phenomena. We have reported that PV serum induces cleavage of Dsg3 in keratinocytes, with subsequent depletion of Dsg3 from cell lysates (Cirillo et al., 2008c). In our experimental conditions, depletion of full-length Dsg3 in PV is associated with fragmentation processes affecting Dsg3. After stimulation of keratinocytes with PV sera, cleavage of Dsg3 occurs within 3 h leading to progressive formation of two fragmentation products with apparent molecular masses of about 60 kDa and 70 kDa. Consistent with the results of Western blotting, analysis of molecular masses of the two fragments has suggested that cleavage did occur intracellularly and resulted from a single cut (Cirillo et al., 2008c). Our findings provide an explanation of the abrogation of Dsg3 from the cell observed by other research groups: Aoyama and Kitajima reported depletion for Dsg3 from Triton-X-100 soluble pool of proteins after incubation of keratinocytes with PV sera, PV IgG (Aoyama and Kitajima, 1999), or anti-Dsg3 IgG (Yamamoto et al., 2007), as mentioned above. We currently do not know whether proteolysis of Dsg3 is part of an acantholytic program taking place after intracellular signals have been generated, or whether it is just the result of the action of an anti-Dsg3 antibody. In the former case, Dsg3 cleavage would be the result of the apoptotic machinery; in the latter, Dsg3 fragmentation would depend on the intracellular degradation

that follows Dsg3 internalization. Further studies are needed to address these questions.

Desmoglein signaling and down-regulation of the desmosomal molecules

It has become clear that induction of acantholysis is an active process that appears to be more complex than the simple interaction of antibodies with adhesion molecules. Cell surface-bound pemphigus IgG causes phosphatidylcholine-specific phospholipase C (PC-PLC) activation, an increase in inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) production, and protein kinase C (PKC) activity (Seishima et al., 1995; Osada et al., 1997); furthermore, there is an increase in intracellular calcium concentration, which appears to result from IP_3-mediated release of intracellular stores (Esaki et al., 1995). Dsg3 is phosphorylated by a kinase other than PKC and appears to dissociate from plakoglobin (Aoyama et al., 1999); this dissociation from adaptor proteins may explain the ability of PV IgG to deplete Dsg3 from desmosomes due to premature internalization. Recently, a role in PV blistering has been demonstrated for a myriad of protein kinases and signaling molecules, including p38 MAPK, PKC, c-myc, Src, RhoA, PERK, FAK, Akt/mTOR, and cdk2 (Waschke et al., 2006; Berkowitz et al., 2006; Williamson et al., 2006; Chernyavsky et al., 2007; Lanza et al., 2008; 2011; Pretel et al., 2009; Cirillo et al., 2010; Gil et al., 2012).

It has been demonstrated in vivo that p38MAPK and one of its downstream targets, heat shock protein (HSP) 27, were phosphorylated in response to PV-IgG and that pharmacologic inhibition of p38MAPK abolished blister formation (Berkowitz et al., 2006). However, the mechanisms by which p38MAPK and HSP27 lead to keratinocyte dissociation are largely unclear. They could involve reorganization of the keratinocyte cytoskeleton but would not affect desmosome directly. In this regard, Sergei Grando reported that activation of p38 MAPK is a late-signaling step associated with collapse of the cytoskeleton and disassembly of desmosomes caused by upstream events involving Src and EGF receptor kinase (Chernyavsky et al., 2007).

It has been shown that PV-induced epidermal splitting, keratinocyte dissociation, and loss of Dsg1/3 binding in vitro were accompanied by inactivation of Rho A. This study suggested that Rho A could be involved in the regulation of desmoglein cytoskeletal anchorage (Waschke et al., 2006), as Rho A activation reduced Dsg3 in the non-cytoskeleton-bound fraction.

Important data reconciling kinase signaling and cell adhesion machinery have been provided by our group. We have shown that phosphorylation events are impaired in PV and that PV sera are able to promote cell cycle progression by inducing the accumulation of cyclin-dependent kinase 2 (cdk2). In this study, cdk2 was shown to control a large number of genes encoding cell adhesion molecules, including desmosomal molecules such as plakophilin 3, desmocollin 2, and desmoplakin (Lanza et al., 2008). Drawing on these findings, we took a systems level approach to the study of cell adhesion in PV by investigating the response of the desmosomal interactome to external perturbations (i.e. PV serum) (Cirillo, 2012). As exemplified in Figure 3, up to 10 out of 22 intrinsic molecules of the desmosomal interactome were transcriptionally down-regulated in the mouse passive transfer model of PV; when we included keratins in the desmo-adhesome, 16 out of 30 molecules showed reduced expression by microarrays (Cirillo and Prime, 2009). More important, pharmacologic inhibition of

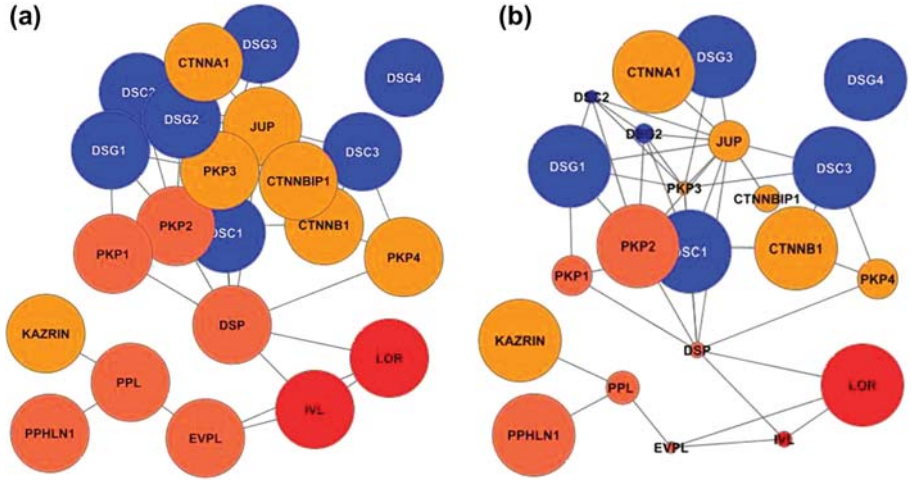

Figure 3. Simplified desmosomal interactome in pemphigus. **(a)** Graphical representation of the intrinsic components of the desmo-adhesome (with the exception of keratins). The intrinsic desmosomal proteins are depicted as circles (nodes) that are connected by lines (interactions) based on their reciprocal binding interactions, thus forming a network. Nodes have been assigned different colors according to the function of the respective proteins: transmembrane (blue), adaptors (orange), and cytoskeleton molecules (red); **(b)** the relative gene expression changes of the same molecules after perturbation of the system in pemphigus vulgaris (PV) have been represented as differential size of the nodes (e.g., down-regulated molecules have proportionally smaller size). The original microarray data are from a mouse model of PV (Lanza et al., 2008).

cdk2 blunted the effect of PV-IgG on the expression of most of the genes, and also blocked PV-IgG-induced acantholysis *in vivo* (Lanza et al., 2008).

Thus, it is conceivable that generalized weakening of desmosomal adhesion is involved in the mechanisms underlying keratinocyte dissociation in pemphigus and that a subset of protein kinases does control this process.

Desmocollins as additional desmosomal antigens with a major role in PV

Again, basic science leads the way of pemphigus research. Desmocollin genes (DSCs) encode N-glycosylated type 1 transmembrane proteins that belong to the cadherin family of calcium-dependent cell-adhesion molecules. To test the hypothesis that Dsc3 functions as a cell-adhesion molecule in adult skin, the group led by Peter Koch assessed the role of DSC3 in the development and maintenance of stratified epithelia in mice using a conditional null allele (Chen et al., 2008). The authors demonstrated that mice with a genetic deficiency of Dsc3 showed a pronounced blistering phenotype with suprabasal loss of epidermal adhesion that is highly reminiscent of PV (Chen et al., 2008). This obviously means that Dsg3 alone cannot sustain epidermal cohesion, thus confuting the key, dogmatic assumption of the desmoglein compensation theory. More important, the data pertaining to Dsc3 function in the epidermis stimulated new ideas and hypothesis on PV pathophysiology that eventually prompted the discovery that patients with typical PV develop anti-Dsc3 antibodies (Mao et al., 2010). As it has been reported that Dsc3, like Dsg3, is preferentially expressed in the basal and suprabasal layers of human epidermis (Chen et al., 2008) - the site where loss of adhesion takes place in PV – it seemed reasonable to speculate that the anti-Dsc3 IgG found in PV sera could participate to the mechanisms of blister formation; the pathogenicity of anti-Dsc3 antibodies was accordingly assessed in subsequent studies. Not surprisingly, it was demonstrated that IgG against Dsc3 caused loss of adhesion of epidermal keratinocytes (Rafei et al., 2011). In this study, IgG against Dsc3 was purified from Dsc3-reactive pemphigus sera by affinity column chromatography using recombinant human Dsc3. Affinity-purified IgG was functionally active and did not only react with recombinant Dsc3 but also with epidermis and cultured human keratinocytes. Moreover, Dsc3-reactive IgG induced loss of adhesion of epidermal keratinocytes in a dispase-based keratinocyte dissociation assay that was reversed on pre-adsorption with human Dsc3 but not Dsg3 (Rafei et al., 2011).

The studies cited above have clearly shown that IgG autoantibodies against a transmembrane component of the desmosome other than Dsg3, namely Dsc3, can induce loss of keratinocyte adhesion and contribute to blister formation in pemphigus.

CONCLUSIONS

The desmosome is the best studied target of pemphigus autoimmunity. Whilst Dsg1/3 have been commonly considered the main players in the pathogenesis of PV, the list of desmosomal molecules involved in PV-induced cell–cell detachment has now increased dramatically. These findings question the supposedly unique role of Dsg1/3 in epidermal physiology and support the concept that PV is a disease of intercellular adhesion as a whole. Furthermore, the intracellular signals that ultimately lead to disruption of cell cohesion may not be triggered by anti-desmosomal antibodies. The evidence supporting this new scenario will be discussed in the second part of the review.

Declaration of interest: The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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