Mechanisms Causing Loss of Keratinocyte Cohesion in Pemphigus

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The autoimmune blistering skin disease pemphigus is caused by IgG autoantibodies against desmosomal cadherins, but the precise mechanisms are in part a matter of controversial discussions. This review focuses on the currently existing models of the disease and highlights the relevance of desmoglein-specific versus nondesmoglein autoantibodies, the contribution of nonautoantibody factors, and the mechanisms leading to cell dissociation and blister formation in response to autoantibody binding. As the review brings together the majority of laboratories currently working on pemphigus pathogenesis, it aims to serve as a solid basis for further investigations for the entire field.

Journal of Investigative Dermatology (2018) **138,** 32–37; doi:10.1016/j.jid.2017.06.022

INTRODUCTION

In recent years, a major debate in pemphigus research has focused on the nature of the autoantigens that are targeted by pathogenic autoantibodies leading to the loss of epidermal cell-cell adhesion. A recent expert meeting (Schmidt et al., 2017) has helped, based on the published evidence and novel data presented, to define an international consensus on how we currently see the immune pathogenesis of pemphigus. This review now centers on the current state of research especially on those aspects of pemphigus pathogenesis that have been a matter of controversy in the past (Ahmed et al., 2016; Amagai et al., 2006).

Since the early studies by Beutner and Jordon (1964), pemphigus has been known to be caused by autoantibodies targeting keratinocyte surface antigens. In line with this notion, the depletion of autoreactive B cells via a CD20-directed antibody is effective in the treatment of patients with pemphigus (Colliou et al., 2013). Specific investigations on disease pathogenesis have been enabled by studies demonstrating that one major autoantigen in pemphigus is desmoglein (Dsg) 3, which belongs to the cadherin superfamily of adhesion molecules (Amagai et al., 1991; Stanley and Amagai, 2006). Identification of desmogleins as targets in pemphigus autoimmunity most recently led to new experimental approaches such as designing Dsg3-specific chimeric autoantibody receptors that may revolutionize therapy in the future (Amagai, 2016; Ellebrecht et al., 2016). Nevertheless, the identification of all targets of pemphigus antibodies together with relevant downstream mechanisms is an important goal to understand the molecular pathways contributing to disease pathogenesis and develop targeted adjuvant therapies. For more information on the diagnosis, treatment, and basic pathophysiology of pemphigus, we may refer to recent comprehensive review articles (Ahmed et al., 2016; Di Zenzo et al., 2016; Hammers and Stanley, 2016; Kitajima, 2014; Kneisel and Hertl, 2011; Spindler and Waschke, 2014; Stahley and Kowalczyk, 2015).

ROLE OF AUTOANTIBODIES DIRECTED AGAINST DESMOGLEIN 1 AND DESMOGLEIN 3

To establish pathogenicity of autoantibodies targeting a particular antigen in pemphigus, the autoantibodies should be shown to be both necessary and sufficient for the loss of cell adhesion. (i) Necessity can be demonstrated by testing the pathogenicity of polyclonal serum IgG after immunodepletion using the antigen of interest, whereas sufficiency can be demonstrated by affinity purification of antibodies against the antigen of interest. However, specificity and efficacy of immunodepletion are not easy to guarantee and monitor. (ii) Antigens can be depleted by knockout or small interfering RNA-mediated knockdown to clarify if the loss of autoantibody-induced function of these antigens is required for pathogenesis. (iii) Antigen-specific monoclonal antibodies purified from patients or animal models of pemphigus can be applied. In pemphigus, the ultimate goal with these approaches is to determine the contribution of autoantibodies

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Abbreviations: Dsg, desmoglein; PV, pemphigus vulgaris

Received 12 February 2017; revised 20 June 2017; accepted 21 June 2017; corrected proof published online 14 October 2017

targeting a particular antigen to the loss of keratinocyte cohesion.

For autoantibodies targeting Dsg1 and Dsg3 in pemphigus vulgaris (PV) and pemphigus foliaceus, the relevance for blister formation has been demonstrated by each of the strategies listed above. Immunoadsorption using recombinant Dsg1 and Dsg3 abolished pathogenic effects in different models in vitro and in vivo (Amagai et al., 1992; Heupel et al., 2008; Langenhan et al., 2014; Mahoney et al., 1999). Dsg3-specific knockout mice show lesions in the mucosa, conjunctiva, and hair follicles, which, on a histological level, resemble lesions in patients with PV (Koch et al., 1997, 1998; Rotzer et al., 2016; Vielmuth et al., 2016), suggesting that anti-Dsg3 antibodies cause the loss of Dsg3 function. Similarly, antibodies targeting Dsg1 and Dsg3 reduce keratinocyte cohesion of murine and human keratinocytes, at least under mechanical stress as in dissociation assays. Antibodies selectively targeting Dsg1 and Dsg3 are sufficient to cause acantholysis and skin vesiculation in mice and in ex vivo human skin (Di Zenzo et al., 2012; Eming et al., 2014; Ishii et al., 2008; Langenhan et al., 2014; Payne et al., 2005; Saito et al., 2012; Spindler et al., 2013; Yamagami et al., 2010; Yeh et al., 2006). PV-IgG have been shown to induce direct inhibition of Dsg3 binding, and several signaling pathways downstream of antibody binding including p38 mitogen-activated protein kinase, Ca^{2+}, protein kinase C, Src, EGFR, RhoA, c-Myc, glycogen synthase kinase 3, Pg, and caspases were shown to be involved in the loss of keratinocyte cohesion in PV, pemphigus foliaceus, and atypical pemphigus (Bektas et al., 2013; Berkowitz et al., 2006, 2008; Caldelari et al., 2001; Chernyavsky et al., 2007; Cirillo et al., 2010, 2014; Dehner et al., 2014; Frusic-Zlotkin et al., 2006; Li et al., 2009; Luyet et al., 2015; Mao et al., 2011, 2014; Saito et al., 2012; Sánchez-Carpintero et al., 2004; Sayar et al., 2014; Spindler et al., 2011, 2014; Waschke et al., 2006; Williamson et al., 2006; Yoshida et al., 2017).

RELEVANCE OF AUTOANTIBODIES TARGETING ANTIGENS OTHERS THAN DESMOGLEINS

In experimental model studies, the concentration of anti-Dsg3 and -Dsg1 autoantibodies is likely substantially higher than the in vivo concentration in patients. This opens the possibility that in patients autoantibodies targeting other antigens may be additionally required to cause disease. A number of cases of acute PV with positive anti-keratinocyte antibodies by direct and/or indirect immunofluorescence but negative Dsg1 and Dsg3 ELISA have been reported, indicating that the level of circulating anti-Dsg antibody is not sufficiently detectable in these cases or that non-Dsg antibodies alone can be responsible for disease development (Belloni-Fortina et al., 2009; Cozzani et al., 2013; Giurdanella et al., 2016; Jamora et al., 2003; Sardana et al., 2013; Sharma et al., 2006; Zagorodniuk et al., 2005). A good although rare example is Dsc3 pemphigus, in which autoantibodies targeting Dsc3, even in the absence of antibodies directed to Dsg1 or Dsg3, have been shown to be pathogenic in vitro and in vivo (Mao et al., 2010; Rafei et al., 2011; Spindler et al., 2009). In line with this, epidermal-specific Dsc3-deficient mice developed a severe PV-like phenotype (Chen et al., 2008). These data collectively provide necessity and sufficiency of antibodies targeting Dsc3, at least in rare cases of PV.

Besides desmosomal cadherins, more than 40 antigens were shown to be targeted by autoantibody fractions from patients with pemphigus including muscarinic and nicotinic acetylcholine receptors, pemphaxin, and mitochondrial proteins (Chen et al., 2015; Lakshmi et al., 2017; Marchenko et al., 2010; Nguyen et al., 2000a). Recently, the formation of autoantibodies against different muscarinic receptors subtypes as well as thyroperoxidase, a protein not known to be expressed by keratinocytes, has been confirmed in an HLA-type-dependent fashion (Sajda et al., 2016). In contrast to autoantibodies targeting Dsg1 and Dsg3, the pathogenic capacity of nondesmoglein antibodies remains unclear. The development of Dsg1- and Dsg3-specific immunoadsorbers appears to be a rational approach for the initial adjuvant treatment of patients with pemphigus with high disease activity (Langenhan et al., 2014). However, it has been shown that autoantibody fractions depleted of autoantibodies against Dsg1 and Dsg3 can be pathogenic and IgG fractions including these antibodies can cause the loss of cohesion under conditions where Dsg3 is not present (Nguyen et al., 2000b). Based on these results, it was proposed that a critical combination of different autoantibodies may be necessary for the development of pemphigus, at least in some subsets of patients. Further functional studies using knockout mice or monoclonal antibodies derived from pemphigus patients or pemphigus mouse models that target a single non-Dsg or non-Dsc antigen are lacking at present, but are required to establish the relevance of non-Dsg/Dsc autoantibodies for pemphigus pathophysiology and clarify their role in the development and/or modification of disease subphenotypes.

CONTRIBUTION OF CYTOKINES AND OTHER FACTORS

Importantly, it has been shown that, besides autoantibodies, cytokines and inflammatory mediators may contribute to blistering in pemphigus including FasL, tumor necrosis factor-α, IL-1β, and IL-6 (Cirillo et al., 2007; Feliciani et al., 2000; Puviani et al., 2003). In particular, PV-IgG-induced caspase 8 activation and Dsg3 cleavage were inhibited by anti-FasL neutralizing antibodies (Grando et al., 2009). However, FasL neutralizing antibodies were unable to reverse changes in cellular elasticity specifically induced by pathogenic, but not nonpathogenic, anti-Dsg3 antibodies (Seiffert-Sinha et al., 2014). Furthermore, it was shown that PV-IgG can stimulate the secretion of cytokines from keratinocytes (Vodo et al., 2016). Expression of the transcription factor ST18 in keratinocytes, which was proposed to account for the different prevalence of pemphigus in certain populations (Sarig et al., 2012), enhanced both secretion of cytokines and loss of keratinocyte cohesion in response to PV-IgG indicating that cytokines can contribute to the pathogenic mechanisms downstream of autoantibodies (Vodo et al., 2016).

MECHANISMS CAUSING BLISTER FORMATION IN PEMPHIGUS IN RESPONSE TO ANTIBODY BINDING

Autoantibody-induced loss of cell-cell adhesion is the cause for skin blistering and mucosal erosions. This phenotype could be explained by the notion that anti-Dsg antibodies

can interfere with the binding of these molecules due to blocking of *trans*- or *cis*-adhesive interfaces (Di Zenzo et al., 2012; Heupel et al., 2008; Tsunoda et al., 2003). However, it is also clear that inhibition of the signaling molecules listed above can prevent the loss of adhesion in pemphigus model systems. This suggests that inhibition of Dsg binding and intracellular signaling cooperate for blister formation. Indeed, there is evidence that signaling occurs at least in part in response to steric hindrance of Dsg interactions and appears to be a prerequisite for full loss of cell cohesion.

Several observations indicate that cellular signaling is important in skin blistering in pemphigus. For example, (i) a number of studies indicate that modulation of signaling pathways can inhibit the loss of cell cohesion and blistering even though pemphigus antibodies are still binding their targets (Berkowitz et al., 2006; Sánchez-Carpintero et al., 2004; Vielmuth et al., 2015; Williamson et al., 2006); (ii) antibodies against Dsg1 were shown to be pathogenic, but no inhibitory effect on the adhesion of Dsg1 molecules could be observed (Waschke et al., 2005); (iii) remaining desmosomes are reduced in size but split when exposed to shear stress (Schulze et al., 2012; Sokol et al., 2015; Stahley et al., 2016; van der Wier et al., 2012); and (iv) on the ultrastructural level, split desmosomes show weakened plaques or altered keratin insertion (de Bruin et al., 2007; Diercks et al., 2009). Observations (iii) and (iv) suggest that reduced desmosomal adhesion and ultimately splitting are downstream of changes in desmosome composition or desmoglein depletion from existing or assembling desmosomes (Aoyama et al., 2010; Oktarina et al., 2011), processes in which signaling should be involved.

Other observations provide support for the idea that at least some pathogenic antibodies directly interfere with adhesion. For example, in one study, the loss of cell-cell adhesion by the monoclonal anti-Dsg3 antibody AK23 could not be blocked by modulating intracellular signaling events (Saito et al., 2012), and p38 mitogen-activated protein kinase activation can also be secondary to the loss of cell cohesion (Mao et al., 2011). One possibility is that the loss of adhesion activates signaling pathways that decrease desmosomal adhesion through uncoupling of keratin filaments, driving desmoglein endocytosis, or dismantling the desmosome plaque. This model predicts a feedforward mechanism in which steric hindrance and activation of signaling act cooperatively to weaken cell-cell adhesive strength.

Indeed, changes in signaling in response to autoantibodies can be caused by the loss of interaction of Dsg3 molecules (Spindler et al., 2013), providing a direct link between steric hindrance and altered signaling patterns. The precise mechanisms by which the loss of Dsg binding alters intracellular signaling are not yet clear, but an association of signaling molecules with desmoglein-based complexes was shown that may contribute to controlling activity states (Rotzer et al., 2015; Tsang et al., 2012). In this regard, specifically the extradesmosomal Dsg molecules may be the first initiators of signaling (Di Zenzo et al., 2016; Müller et al., 2008; Sayar et al., 2014), either because they are more easily accessible by autoantibodies compared with the molecules densely packed in the desmosome or because they have additional functions in controlling intracellular signaling, which in turn may impair desmosome function.

A main consequence of pemphigus autoantibody binding is the altered turnover of desmosomal adhesion molecules. Dsg disassembly is enhanced in a signaling-dependent manner (Jolly et al., 2010; Saito et al., 2012). Specifically, the linear arrays and clusters detectable in response to autoantibody application (Oktarina et al., 2011) may represent finger-like protrusions enriched in desmosomal cadherins that serve as sites of internalization (Jennings et al., 2011; Sokol et al., 2015). The reduced desmosome assembly observed in response to pemphigus autoantibodies likely results from the rapidly reduced extradesmosomal pool of desmosomal cadherins, thereby depleting the assembly pool of desmogleins available for incorporation into desmosomes (Mao et al., 2009; Oktarina et al., 2011; Williamson et al., 2006; Yamamoto et al., 2007). Desmosomal molecules localize to lipid rafts, the latter of which are required for desmosome assembly and for disassembly following pemphigus autoantibody binding (Stahley et al., 2014). Indeed, both Dsg3 and Dsg1 interact with the lipid raft markers flotillin-1 and -2, the expression of which is important for the membrane localization of the two desmosomal cadherins (Vollner et al., 2016). Finally, intermediate filament uncoupling from the desmosomal complex in response to pemphigus autoantibodies (keratin retraction) may contribute to the loss of cell cohesion by affecting desmosome protein turnover in a signaling-dependent manner, as indicated by experiments with keratin-deficient keratinocytes (Kröger et al., 2013). Additional factors such as cytokines or mitochondrial damage may contribute to the pemphigus phenotype by increasing the susceptibility of keratinocytes for changes in signaling and desmosome turnover (Kalantari-Dehaghi et al., 2013; Vodo et al., 2016).

OUTLOOK

Major advances have been made in the understanding of pemphigus pathogenesis during the last decade. With respect to both factors and mechanisms causing loss of keratinocyte cohesion and blistering, the picture is getting more and more detailed but also complex. The novel insights need to be further clarified but should also be vigorously applied to identify additional treatment options. For instance, the observation that steric hindrance and signaling may be linked makes the pharmacologic targeting of signaling molecules an attractive approach. Novel studies using suitable translational models are needed to carefully characterize the effects of pharmacologic approaches with respect to the delicate balance of signaling patterns essential for epidermal homeostasis. We hope that this consensus statement can serve as a valid basis for future investigations and further fruitful discussions.

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CONFLICT OF INTEREST

ASP is a consultant for Syntimmune and inventor on a patent for the chimeric autoantibody receptor technology.

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