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Atypical pemphigus: Autoimmunity against desmocollins and other non-desmoglein autoantigens

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ABSTRACT

In this review, we recap current knowledge about non-desmoglein autoantigens in atypical forms of autoimmune pemphigus. More than 50 keratinocyte proteins, including adhesion molecules, receptors and enzymes as well as mitochondrial proteins can be targeted, leading to alterations in numerous intracellular signaling pathways. Patients with pemphigus herpetiformis feature various combinations of antibodies to desmogleins 1 and 3 and desmocollins 1-3. Pemphigus vulgaris patients who do not have antibodies to desmogleins develop typical clinical and histological features of pemphigus. Experimental results revealed synergy of different autoantibodies. Alterations of the keratinocyte adhesive function caused by a single antibody alone are reversible due to self-repair. Since composition of the pool of the most common pathogenic antibodies appears to be similar among pemphigus patients with or without anti-desmoglein antibodies, the atypical pemphigus represents a unique model for elucidation of the molecular mechanisms of autoimmunity against non-desmoglein antigens. Further studies of the immunopathology of atypical pemphigus should shed new lights on the pathophysiology of conventional variants of autoimmune pemphigus.

Introduction

Autoimmune pemphigus comprises a group of autoantibody (**AuAb**)-mediated blistering diseases caused by IgG molecules directed against keratinocyte components resulting in a loss of cell-cell adhesion and acantholysis. Typical pemphigus is classified in two main forms: pemphigus foliaceous (**PF**) and vulgaris (**PV**), in which AuAbs recognize desmosomal proteins desmoglein 1 (**Dsg1**) and desmoglein 3 (**Dsg3**). Many efforts have been made to clarify mechanisms of autoimmune response in pemphigus, and different models have been developed during the last decades. Discussion of the well-known explanation of PV and PF based on an interplay of anti-Dsg1 and anti-Dsg3 AuAbs is beyond the scope of this paper. The strengths and weaknesses of the simplistic explanation of pemphigus immunopathology have been recently evaluated by international experts in pemphigus [1].

In consequence of the growing body of evidence concerning the anti-Dsg1/3 AuAb-independent phenotypes, the studies focused on new potential targets gave a decisive boost to the development of alternative explanations of the disease mechanisms [1]. To date, more than 50 non-Dsg1/3 target antigens have been identified. These form a heterogeneous group, including various adhesion proteins, acetylcholine receptors, mitochondrial proteins, thyroid peroxidase, peripheral myelin protein 22, human leukocyte antigen proteins and the secretory pathway Ca^{2+}/Mn^{2+}-ATPase isoform 1 (**SPCA1**) as well as some other keratinocyte proteins whose roles in pemphigus pathophysiology have not been yet elucidated. Correspondingly, alterations in numerous signaling pathways have been associated with the binding of AuAbs directed against keratinocyte antigens other than Dsg1/3 (reviewed in [2]). Recent data from in vivo and in vitro experiments suggest synergistic roles of non-Dsg AuAbs in pemphigus pathogenesis, but more studies are required to identify exact contribution of each AuAb type.

Herein, we briefly recap current knowledge concerning the non-Dsg1/3 target antigens in both typical and atypical forms of autoimmune pemphigus. The detailed discussions of non-Dsg1/3 AuAbs in patients with typical forms of pemphigus can be found in previously published works (eg, [3, 4]).

Autoantibodies to non-Dsg1/3 targets in patients with typical forms of PV and PF

AuAbs to keratinocyte adhesion molecules

In a study of a large cohort of pemphigus patients, AuAbs to desmocollin (**Dsc**) 1 and Dsc3 were present in 44% of patients, with only 7% in matched controls [5]. In a study of 164 pemphigus patients, anti-Dsc AuAbs were found in 3 out of 22 PV cases (13%) and 3 out of 18 PF cases (18%) [6].

The proteomics analysis of a large number of PV patients demonstrated that 43% of patients had AuAbs targeting plakophilin 3, compared to 7% of matched controls [5].

Plakoglobin has been precipitated by sera of PV and PF patients in associated with Dsg molecules [7]. It is internalized in combination with Dsg3, which is believed to cause retraction of keratin filaments [8]. In another study, PV IgG appeared to promote separation of Dsg3 and plakoglobin [9]. Proteomics studies demonstrated that 26% of PV patients carried an AuAb against junctional plakoglobin, compared to 5% of controls [5].

The AuAb targeting E-cadherin also has been found in pemphigus sera [10, 11]. Approximately 78% of patients with mucocutaneous PV and 33% of patients with mucosal PV featured this AuAb.

AuAbs to keratinocyte receptors and enzymes

AuAbs against both the muscarinic and the nicotinic classes of cholinergic (acetylcholine) receptors (**AChRs**) expressed on keratinocytes have been identified in pemphigus patients

(reviewed in [12]). Noteworthily, blocking of either class of keratinocyte AChRs leads to disassembly of desmosomal and adherence junctions due to phosphorylation of desmosomal and classical cadherins, respectively, whereas cholinergic agonists prevent cell detachment by activating protein phosphatases and upregulating expression of the cadherin genes.

The protein array technology identified M_1, M_2, M_4 and M_5 muscarinic AChR subtypes as targets of pemphigus autoimmunity [13, 14]. Lakshmi et al [15] found that anti-M_3 mAChR (**M3AR**) AuAb is present in 100% of PV patients.

Targeting of α3 subunit of the nicotinic class of keratinocyte AChRs was first discovered in a patient with coexistent PF, myasthenia gravis and thymoma [16]. Reactivity with α9 subunit was observed in the immunofluorescence blocking experiments, wherein staining of monkey esophagus by rabbit anti-α9 AuAb was prevented due to preincubation of the substrate with PV antibodies [17]. The proteomics approach demonstrated reactivity with α10 subunit that can be a part of the pentameric α9α10 nicotinic AChR. The immunoprecipitation-western blot assay of keratinocyte mitochondrial proteins visualized the α3, α5, α7, α9, α10, β2 and β4 subunits of nicotinic AChR precipitated by PV IgGs, indicating that the anti-apoptotic function mitochondrial nicotinic AChRs is compromised in PV [18].

Probing of keratinocyte λgt11 cDNA library with PV IgGs eluted an AuAb from a 75 kD band that stained epidermis in pemphigus-like intercellular pattern and caused acantholysis in the keratinocyte monolayers, thus revealing a novel type of AChRs termed pemphaxin (a.k.a. annexin 9) [19].

The analysis of sera from 28 PV patients by ELISA revealed that 39% had AuAb targeting the α chain of the high-affinity receptor for the Fc region of IgE [20]. The significance of the of this type of AuAb in pemphigus remains unclear.

Cellular enzymes are also targeted by pemphigus autoimmunity. The association between PV and anti-thyroid peroxidase AuAb has been documented in several studies [21-23], but its connection to pemphigus pathophysiology remains to be elucidated. The secretory pathway Ca^{2+}/Mn^{2+}-ATPase isoform 1 (**SPCA1**) is targeted in 43% of patients with PV compared to 8% in matched controls [5]. The significance of this AuAb to pemphigus pathophysiology will be discussed later in this text.

AuAbs to mitochondrial proteins

Mitochondrial autoimmunity was a missing link in PV pathophysiology, because: 1) anti-mitochondrial AuAbs (**AMA**) launch the intrinsic apoptotic pathway, which has been documented in keratinocytes exposed to PV IgGs, 2) adsorption of AMA abolishes the ability of PV IgGs to cause acantholysis both *in vitro* and *in vivo* and 3) at the physiological concentration, anti-Dsg AuAb requires the presence of AMA to induce acantholysis [24]. The proteomic analysis of antigenic reactivities of PV IgGs from 264 PV patients *vs.* 138 healthy donors against 701 keratinocyte proteins spotted on microarray demonstrated that 88% of PV patients have antibodies to at least one mitochondrial protein [5]. The mitochondrial proteins targeted in PV patients with the frequency that exceeded that in controls by 10 and more times includes NADH-ubiquinone oxidoreductase, cytochrome b5 outer mitochondrial membrane isoform precursor, superoxide dismutase, subunit of pyruvate dehydrogenase E1 component and fumarate hydratase [25]. Chen et al [24] demonstrated that PV IgGs including AMA couple with neonatal Fc receptor (**FcRn**) on the cell membrane. The PV IgG-FcRn complex allows the entrance of AMA to the keratinocyte. Once in the cytosol, they dissociate and are trafficked to the mitochondria where they trigger pathologic events [18, 26]. Based on the known functions of targeted proteins, the following mitochondrial pathways might be subject to dysfunction: tricarboxylic acid cycle, oxidative phosphorylation, O_2 respiration, and production/inactivation of ROS. Indeed, AMA produced by PV patients disrupt the electron transfer chain, resulting in a loss of electrochemical gradient across the inner membrane, increase ROS production and

reduce the ability of keratinocytes to respond to stress [25]. Such abnormalities can trigger the cell death signalling cascade, wherein the executioner caspases cleave selected cell adhesion molecules, such as desmosomal cadherins, leading to both cell detachment (acantholysis) and death (apoptosis) — the unique form of keratinocyte demise in PV termed apoptolysis [2].

Pemphigus Herpetiformis

In 1955 Flodel and Gentele coined the definition “dermatitis herpetiformis with acantholysis” for a clinical phenotype resembling herpetiform dermatitis clinically and PV immunopathologically [27]. Since 1975, when Jablonska et al [28] introduced the term “pemphigus herpetiformis (**PH**)” and proposed diagnostic criteria, about a hundred of cases of PH have been reported. PH does not present predilection for race or gender and its age of onset is highly variable, with pediatric cases occasionally reported [29]. Clinically, it affects the trunk, buttocks and limbs with annularly arranged blisters or grouped vesicles on erythematous skin accompanied by intense itch. Unlike typical pemphigus, mucosal erosions are rare and can arise only secondarily during the disease course. Spongiosis and a dense inflammatory infiltrate composed by neutrophil and eosinophil granulocytes are predominant histopathological findings in PH. Acantholysis is not observed in most patients and, when present, is detectable only in a few areas [28]. Interestingly, the possibility that clinical presentation may shift towards typical PV has been reported [30]. Compared to typical PV, PH does not represent a life-threatening condition, has an indolent course in most patients and is generally well controlled by systemic corticosteroid therapy.

Direct immunofluorescence (**DIF**) highlights IgGs with/without C3 throughout superficial layers of epidermis. Indirect immunofluorescence (**IIF**) shows IgGs to epithelial cell surface [30]. A number of PH patients with anti-keratinocyte IgA AuAbs have been reported as well (reviewed in [31]). Recent advances in diagnostic techniques facilitate characterization of a wide spectrum of distinct immunopathological phenotypes of PH. The PH patients feature various combinations of IgG AuAbs to Dsg1, Dsg3, Dsc1, Dsc2 and Dsc3. For instance, Hong et al [32] reported a case of PH with only IgG to Dsc3. Tateishi et al [33] presented a case with anti-Dsc1 AuAb without Dsg1/3 AuAbs. Kozlowska et al [34] described a patient with clinical and histologic features of PH associated with IgG and IgA AuAbs to Dsg1 and exclusively IgG AuAb to Dsc3. Matsukura et al [35] reported a case of PH with high titer of IgG AuAb to Dsg1 who also had IgG AuAb to Dsc3. Ohata et al [36] described a case of PH with IgG AuAb to Dsc1 coexisting with bullous pemphigoid and AuAb to BP180 C-terminal domain and laminin γ2.

The clinical-pathological peculiarities of PH may be explained by contribution of target antigens other than Dsg1/3 and/or specific autoreactive T or B cell response to endogenous epitopes distinct from the disease-inducing epitopes. Data from animal models of autoimmune diseases confirm that targets of autoimmune response do not remain fixed, and this phenomenon might play a significant role in the clinical variability of PH [37]. In particular, AuAbs against structural proteins other than Dsg are the most likely suspect to account for the clinical heterogeneity of PH. Dsc 1-3 display some characteristics similar to the Dsg group and play an important role in intercellular adhesion. The extracellular portion of Dsc molecules mediates cell-cell adhesion, whereas their intracellular portion connects with the intermediate filament net and constitutes part of desmosomal plaque. Indeed, the presence of both anti-Dsc3 and anti-Dsg3 AuAbs leads to a more severe phenotype and a slower response to prednisolone in experimental pemphigus in mice [38].

Non-desmoglein Pemphigus

<u>Evidence that non-Dsg AuAbs are pathogenic</u>

PV patients who do not have AuAbs to Dsg1 and Dsg3 develop typical clinical and histological features of autoimmune pemphigus and feature positive DIF and IIF, but ELISA results for Dsg1/3 AuAbs are negative. On average, 10-15% of acute PV patients are negative for anti-Dsg1/3 AuAbs [39-45]. Since the immunopathologic mechanisms of acantholysis in these PV patients may be different, such "non-Dsg PV" (the term coined by Chernyavsky et al [46]) is considered to be atypical. Thus far, the best studied self-antigens in non-Dsg PV are Dsc3, M3AR and SPCA1 [47]. The levels of AuAbs against these molecules in PV patients' serum correlate with the stage of disease. These AuAbs are pathogenic, because 1) adsorption of IgG serum fraction from patients with non-Dsg PV with recombinant Dsc3, M3AR, and SPCA1 prevents spontaneous skin blistering in the passive transfer PV model in neonatal BALB/c mice and 2) their transfer to neonatal BALB/c mice causes the appearance of Nikolskiy sign on mouse skin. Furthermore, the acantholytic activity of pre-absorbed PV IgGs can be restored by adding the eluted AuAbs [47]. Similar results were observed in the in vitro models of PV — the neonatal mouse skin explant and the 3D organ culture of human epidermis [46, 47]. However, when given alone, neither of these AuAbs induce acantholysis on their own, suggesting that a simultaneous hit by a pool of non-Dsg AuAbs to different self-antigens is required to disrupt the integrity of epidermis.

Evidence that anti-Dsc3 AuAb is pathogenic in non-Dsg PV

The anti-Dsc3 AuAb was first demonstrated in PV patients more than two decades ago [48, 49]. Anti-Dsc3 AuAb has been shown to be pathogenic in PV patients [50, 51]. Importantly, the *Dsc3*$^{fl/fl}$/K14-Cre mice lacking Dsc3 develop PV-like phenotype [52]. Findings that adsorption of anti-Dsc3 AuAb abolishes acantholysis and skin blistering in neonatal mice [47] extents previous observations made in in vitro experiments. In a case report of a 55 year old female with non-Dsg PV affecting gingival mucosa, adsorption of anti-Dsc3 AuAb with the recombinant extracellular domain of Dsc3 abolished acantholytic activity of patient's IgGs in a primary human keratinocyte cell dissociation assay [50]. And the other way around, a monoclonal AuAb targeting the extracellular domain of Dsc3 caused intraepidermal blistering in an vitro model of human skin, and a loss of intercellular adhesion in cultured keratinocytes [53]. Recent studies with adaptive transfer of autoimmunity to Dsc3 in the Rag2$^{-/-}$ immunodeficient mice demonstrated that anti-Dsc3 AuAb is sufficient to induce pemphigus phenotype [38].

Evidence that anti-M3AR AuAb is pathogenic in non-Dsg PV

Although the presence of AuAbs against keratinocyte muscarinic AChRs in PV patients has been known for about 25 years [54], specific targeting of M3AR was discovered only recently in proteomic studies performed independently by two different groups [5, 55]. It has been demonstrated that the titer of anti-M3AR AuAb correlates with disease activity of PV and it declines with therapy [15, 47]. M3AR is predominantly expressed in the lowermost epidermal layer wherein it regulates vital function of keratinocytes such as proliferation, migration, and cell-cell and cell-substrate attachments (reviewed in [56, 57]). The pathogenic activity of anti-M3AR AuAb apparently stems from phosphorylation of classical and desmosomal cadherins in keratinocytes, by analogy with that observed upon functional inactivation of M3AR through the pharmacological approach (reviewed in [57]).

The role for autoimmunity against keratinocyte M3AR in non-Dsg PV was evaluated using 1-day old M3AR$^{-/-}$ mice [46]. Compared to wild-type (**WT**) mice, the intact M3AR knockout mice demonstrated an increase of intercellular spaces due to mild shrinkage of epidermal cells. Similar subtle morphological changes were observed in WT pups injected with the affinity-purified anti-M3AR AuAb. Injection of PV IgGs caused spontaneous skin blistering and superficial erosions in both WT and M3AR$^{-/-}$ mice. However, while injection of PV IgGs to a WT mouse predictably caused suprabasal acantholysis, the basal cell layer was missing at the bottom of blister cavity in M3AR$^{-/-}$ mice. The disappearance of the basal cells in blisters induced

by PV IgGs in M3AR$^{-/-}$ mice suggested that in non-Dsg PV, anti-M3AR AuAb determines the suprabasal location of the intraepidermal split. The physiologic stimulation of keratinocyte M3AR with endogenous acetylcholine maintains adhesion of basal cells to the basal membrane by upregulation of sedentary (hemidesmosomal) integrins anchoring keratinocytes to the basal membrane [58, 59]. Since the basal cells remained attached to the basal membrane of WT mice treated with PV IgGs, it is likely that anti-M3AR AuAb exhibits an agonist-like immunopharmacologic effect, by analogy with the thyrotropin receptor AuAb that stimulates the thyroid function in Graves' hyperthyroidism.

Evidence that anti-SPCA1 AuAb is pathogenic in non-Dsg PV

An important role of SPCA1 in the physiologic control of keratinocyte adhesion is illustrated by the fact that mutation the *ATP2C1* gene encoding SPCA1 causes Hailey–Hailey disease (also known as familial benign chronic pemphigus) representing a non-immune phenocopy of cutaneous lesions in PV [60, 61]. Keratinocytes from these patients show evidence of both early apoptosis [62] and abnormal Ca^{2+} signaling [63]. In other cell types, suppression of SPCA1 affects cell survival, disturbs Ca^{2+} homeostasis, and causes embryonic lethality [64]. Specific signs of apoptosis are observed in experimental models of SPCA1 deficiency [64, 65]. The anti-SPCA1 PV AuAb causes shrinkage of keratinocytes in 3D organ culture of human epidermis [46], which may represent activation of the cellular apoptotic program. Indeed, anti-SPCA1 AuAb can activate caspase-2 (**Cs-2**) in keratinocytes and induce the cytochrome c (**CytC**) release abolishable by Cs-2 inhibitors [46], suggesting the pivotal role of Cs-2 in the pro-apoptotic effects of anti-SPCA1 AuAb in non-Dsg PV.

While the Dsc3 and M3AR molecules are located on the cell surface of keratinocytes wherein they are easily accessible to AuAbs, the mechanism allowing PV AuAbs to find their way to the intracellular self-antigens, such as SPCA1, is not fully understood. SPCA1 might be reached by an AuAb in a complex with FcRn that has been shown to bind PV IgGs on the cell membrane of keratinocytes and traffic them to intracellular targets [24]. Indeed, commercial anti-FcRn antibody decreases CytC release induced by anti-SPCA1 AuAb, and this effect could not be abolished by the lysosomal enzyme inhibitors chloroquine and monensin [46]. These results demonstrate that FcRn contributes to the pathogenic action of anti-SPCA1 AuAb, in part, by enabling its internalization and intracellular trafficking to the Golgi apparatus wherein SPCA1 is located.

The role of Cs-2 in mediating the pathobiologic effects of anti-SPCA1 AuAb in non-Dsg PV is of particular interest, because it can link Golgi stress to the mitochondrial permeability transition pore (**mPTP**) opening via several mechanisms reviewed elsewhere [66]. It is well established that the Golgi-dependent apoptotic signaling pathways capable of triggering the mitochondrial (intrinsic) apoptosis due to mPTP opening with subsequent CytC release can be mediated by Cs-2 [67]. Cs-2 can stimulate CytC release by facilitating mitochondrial outer membrane permealization both independently of the Bcl-2 family protein, such as Bax, Bak and Bcl-2 [68], and upon direct interaction with Bcl-2 family proteins [69], eg, via cleavage of Bid [70]. Noteworthily, PV IgG binding to keratinocytes has been shown to upregulate Bax and downregulate Bcl-2 expression, thus increasing the Bax/Bcl-2 ratio [71].

Molecular synergy among pathogenic anti-Dsc3, anti-M3AR and anti-SPCA1 AuAbs in non-Dsg PV

The need to combine anti-Dsc3, M3AR and SPCA1 AuAbs to reproduce the acantholytic effect of PV IgGs in vitro and in vivo, suggested a synergy of the pathogenic actions of these AuAbs [47]. While a single type of AuAb can on its own alter keratinocyte function, the resulting changes are insufficient to produce disease phenotype due to self-repair. Therefore, different non-Dsg AuAbs need to concur to impose a simultaneous hit on keratinocytes affecting the

intracellular homeostasis to the degree that it can no longer restore the natural ability of keratinocytes to maintain the integrity of epidermis owing to self-repair.

By analogy with a proposed mechanism of acantholytic action of the AuAb against Dsg3 [72], the mechanism of acantholytic action of anti-Dsc3 AuAb may involve steric hindrance at the site of desmosome, wherein this AuAb can interfere with desmosome formation. However, intact desmosomes are not permeable to AuAbs. The initial event of acantholysis is basal cell separation from each other and immediate suprabasal keratinocytes due to their shrinkage [73]. Basal cells shrink due to collapse and retraction of the tonofilaments cleaved by executioner caspases and dissociation of intradesmosomal adhesion complexes caused by phosphorylation of adhesion molecules. When basal cells shrink, the stretching of their plasma membrane areas anchored to neighboring cells via desmosomes tears them off. Keratinocyte shrinkage, however, is reversible due to self-repair. During recovery, keratinocytes extend their cytoplasmic aprons toward neighboring cells. This is when anti-Dsc3 AuAb can prevent assembly of nascent desmosomes due to steric hindrance, thus rendering acantholysis irreversible. The unique paradigm of keratinocyte separation in PV is termed apoptolysis, which describes the linkage of basal cell shrinkage and activation of cell death pathways rendering a 'tombstone' appearance to PV lesions [2]. Apoptolysis is a complex process initiated in non-Dsg PV by at least three types of AuAbs directed against keratinocyte proteins that mediate intercellular adhesion, ie, Dsc3, control vital cell functions, ie, M3AR, and regulate cell viability, ie, SPCA1.

Thus, atypical pemphigus represents a unique model for elucidation role of AuAbs to non-Dsg antigens in the physiological regulation of keratinocyte cell-cell adhesion and blister development. Since the DIF/IIF patterns of both anti-Dsg AuAb-positive and -negative PV patients are the same, it is obvious that non-Dsg AuAbs contribute to the "intercellular", ie, fishnet- or chicken wire-like, staining pattern produced by serum IgGs from patients with typical pemphigus. If autoimmunity to Dsg3 and Dsg1 represented the only kind of anti-keratinocyte AuAbs in patients with mucosal PV and PF, respectively, as the "Dsg compensation hypothesis" postulates [74], then the DIF and IIF would localize anti-keratinocyte IgGs to the lowers and upper epidermal compartments, wherein their respective self-antigens are predominantly expressed [75]. However, it is well-known that anti-keratinocyte AuAbs from patients with mucosal PV (supposedly represented by anti-Dsg3 AuAb alone), mucocutaneous PV (supposedly represented by a combination of anti-Dsg3 and anti-Dsg1 AuAbs) and PF (supposedly represented by anti-Dsg1 AuAb alone) decorate the entire epidermis producing identical staining patterns. Since composition of the pool of the most common non-Dsg AuAbs appears to be similar among PV patients with or without anti-Dsg AuAbs, ie, both in typical and atypical pemphigus, elucidation of the mechanisms of pathogenic actions of non-Dsg AuAbs in atypical PV should shed new lights on the immunopathology of conventional variants of autoimmune pemphigus.

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