APOPTOTIC PATHWAYS IN THE PATHOGENESIS OF PEMPHIGUS: TARGETS FOR NEW THERAPIES

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Abstract

Pemphigus is a group of rare autoimmune blistering diseases of the skin in which autoantibodies to desmosome cadherins, desmogleins, induce loss of cell-cell adhesion (known as acantholysis). In addition to steric hindrance and activation of intracellular phosphorilation cascade signaling pathways, apoptosis has been suggested to contribute to the mechanism by which pathogenic IgG induces acantholysis. We review the current literature examining the role of apoptosis in pemphigus. Current data recognized a central role of apoptosis in blister induction mechanisms. In particular here we review the pivotal role of FasL in pemphigus, as molecule able to induce both apoptosis and acantholysis. Being pro-apoptotic molecules important in blister formation, they could represent new specific targets for pemphigus treatment.

Keywords: acantholysis, antibodies, apoptosis, caspases, desmoglein, FasL, pemphigus

WHAT IS PEMPHIGUS?

Pemphigus is a group of rare chronic IgG-autoantibody-mediated diseases of the skin and mucosa characterized by intraepidermal blister formation, resulting in the clinical appearance of flaccid bullae and non-healing erosions. Pemphigus is caused by loss of epidermal cells cohesion and detachment of keratinocytes, termed acantholysis [1]. Pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are the two main variants. In these disorders, autoantibodies are mainly directed to desmosomal cadherins. In PF, pathogenic IgG target the desmosome cadherin desmoglein 1 (dsg1) [2], inducing loss of adhesion in the subcorneal region of epidermal; whereas, in PV, antibodies to desmoglein3 (dsg3) in mucosal PV [3] and to dsg3 and dsg1 in mucocutaneous PV [4] induce loss of adhesion in the suprabasal layer. Despite definitive statistics on the incidence and prevalence are not available, the International Pemphigus Foundation registry can estimate 0,1-5 new diagnoses each year per one hundred thousand of people worldwide. The variability depends upon the type of pemphigus and the ethnicity of the affected population (www.pemphigus.org). Therapeutic strategies are mainly directed against the formation of autoantibodies through systemic administration of high dose corticosteroids and/or other immunosuppressive drugs. Most patients need chronic treatment to keep the disease under control, leading to severe side effects, often causing high mortality rate (5-15%) [5].

The mechanism of cell detachment and blister formation is a matter of intensive research [6]. Elucidation of the pathophysiologic mechanisms of acantholysis will facilitate development of novel pharmacologic approaches to prevent and treat pemphigus.

PATHOMECHANISMS OF BLISTER FORMATION IN PEMPHIGUS

Pathogenic autoantibodies in pemphigus sera (PVIgG) target molecular components of the desmosomes, dsg1 and dsg3 [7], while a pathogenic relevance for non-desmoglein antibodies has also been reported, such as antibodies against pemphaxin [8], alpha 9 acetylcholine receptor [9] and mitocondrial antigens [10]. Although autoantibodies play an essential role in the pathogenesis of

pemphigus, the mechanisms leading to the formation of the blister remain largely unknown. PVIgG do not directly split desmosomes, and binding to antigen *per se* does not inhibit desmosome formation [11]. In addition, desmosomes form morphologically normal in dsg3 knockout mice [12]. On the other hand, PVIgG, upon binding to keratinocytes, induce a rapid transient increase of intracellular Ca^{2+} [13] and induce a number of keratinocyte signaling pathways that have emerged as important components in pemphigus acantholysis. PVIgG phosphorylate dsg3 [14], activate Src [15] and induce phosphorylation of p38 mitogen-activated protein kinase (MAPK) in vitro and in vivo [16; 17]. Moreover, PVIgG induce clustering, internalization and intracellular pathway activation of epidermal growth factor receptor (EGFR). Indeed, inhibition of EGFR prevents acantholysis in vitro [18]. Moreover an imbalance of Akt/mTOR has been reported in the passive transfer pemphigus mouse model [19]. Pre-treatment of neonatal mice with Src inhibitor blocks PVIgG-induced acantholysis, thus validating the conclusion that activation of the EGFR/Src cascade in a crucial early event in PVIgG signaling [20]. Downstream mechanisms such as alteration of desmosome assembly [21] and reorganization of the cytoskeleton [22] following binding of PVIgG to the keratinocyte surface receptors have also been described.

Several lines of evidence indicate that apoptosis is involved in the pathomechanisms of pemphigus [20]. Puviani and colleagues first identified the presence of TUNEL positive cells in pemphigus patients' skin before keratinocyte detachment [23], while Pacheco-Tovar *et al.* found TUNEL positive cells in the roof and in the floor of pemphigus blister [24]. Moreover apoptotic cells were detected also in passive transfer pemphigus mouse model skin [19; 25]. PVIgG and pemphigus sera are able to activate caspases in cultured keratinocytes [23; 26; 27], to induce increased expression of pro-apoptotic molecules and depletion of anti-apoptotic proteins [26; 28].

In addition to autoantibodies, pemphigus serum contains the non-IgG substances that can cause a sharp reduction of keratinocyte viability and weaken intercellular adhesion strength [29]. The serum factors implicated in pemphigus pathomechanisms include Fas Ligand (FasL) [23], tumor necrosis factor (TNF) alpha [30], nitric oxide [31], kallikreins, and other proteases [32].

APOPTOSIS

Apoptosis is a specific type of cell death. It is characterized by rounding-up of the cell, retraction of pseudopodes, reduction of cellular volume (pyknosis), chromatin condensation, nuclear fragmentation (karyorrhexis), classically little or no ultra structural modifications of cytoplasmic organelles, plasma membrane blebbing (but maintenance of its integrity until the final stages of the process) and engulfment by resident phagocytes (in vivo) [33]. There are several distinct subtypes of apoptosis that, although morphologically similar, can be triggered through different biochemical routes, for example the intrinsic and extrinsic pathways [34]. The extrinsic apoptotic pathway is classically mediated by death receptors, including Fas and other TNF-receptor family proteins. Death receptors rely on signaling proteins possessing a distinct set of modular protein motifs capable of homo-typic interaction, including death domains (DD) and death effector domains (DED) [35; 36]. In particular, Fas, which is preassembled as a trimer, undergoes conformational change following FasL binding and assembles on its cytoplasmic tail a signaling complex known as the DISC (Death-Inducing Signaling Complex) [37]. The adaptor FADD/MORT, bearing both DD and DED motifs, binds the DD of Fas and recruits procaspase-8 via its DED domain [38]. Activation of caspase-8 in the DISC complex is believed to follow an "induced proximity" where high local concentration of procaspase-8 leads to its auto-proteolytic activation and subsequent activation of caspase-3 and -7 [39].

At the skin level, Fas is expressed at the membrane level by all basal and immediately suprabasal keratinocytes [40]. Whereas FasL is confined to basal and first suprabasal layers of the epidermis, homogeneously distributed within the cytoplasm and without plasma membrane enhancement. By electron microscopy analysis, FasL localization is almost exclusively confined to the cytoplasm, mostly in association with intermediate filaments [41].

The Fas system has proven to be essential in contributing to the functional integrity of the epidermis. Evidences have shown that Fas-induced keratinocyte apoptosis in response to UV prevents the accumulation of pro-carcinogenic p53 mutations by deleting UV-mutated keratinocytes

[42]. Furthermore, strong evidence exists that dysregulation of Fas expression and/or signaling contributes to the pathogenesis of diseases, such as toxic epidermal necrolysis [43], acute cutaneous graft versus host disease [44; 45] and pemphigus.

FasL AND PEMPHIGUS

An important role of Fas system in pemphigus pathomechanisms is well documented. Original immunohistochemical studies revealed Fas receptor on keratinocytes membranes of pemphigus lesional epidermis [46]. It has been demonstrated that sera from untreated pemphigus patient contain highest level of FasL as compared to sera from healthy subjects. After 2 weeks of steroids treatment, FasL levels decrease to healthy serum levels [23]. In the same work, Puviani and colleagues first demonstrated that FasL contained in pemphigus sera induce apoptosis in normal human keratinocytes, via caspase-8 activation [23]. In addition, there are evidences that PVIgG treatment induce an mRNA up-regulation of pro-apoptotic molecules in keratinocytes, including FasL [28] and a secretion from cells of soluble FasL [27]. This appears to be not just a mere up-regulation, as PVIgG treatment induces a co-aggregation of FasL and Fas receptor with caspase-8 in a with DISC formation [27]. Using an organ culture model of pemphigus, it has been demonstrated that FasL acts synergistically with PVIgG and TNF alpha in the induction of acantholysis [47]. Neutralization of FasL prevents caspase-8 activation and decreases the number of apoptotic cells after treatment of keratinocytes with pemphigus sera [23]. It has been shown that caspases may directly cleave desmogleins and other adhesion molecules, leading to desmosome destruction [48; 49]. Indeed, when PVIgG are added to keratinocytes in the presence of anti-FasL neutralizing antibody, the cleavage of the intracellular portion of Dsg3 and its degradation decreases, preventing the PVIgG-induced acantholytic effect. Moreover, caspase-8 is not activated in presence of PVIgG and anti-FasL neutralizing antibody [20]. Also caspase-8 inhibitor prevents apoptosis in keratinocytes treated with pemphigus sera [23].

Iordanov *et al.* have demonstrated that FasL is able to trigger a profound phosphorilation of EGFR and of its downstream effectors ERK and protein kinase B (PKB/Akt). Using a variety of inhibitors and blocking antibodies, they demonstrated that: (i) apoptosis is required for the generation of the signal(s) leading to the activation of EGFR, ERK, and Akt; (ii) the activation of EGFR, ERK, and Akt by FasL is indeed mediated by its *bona fide* receptor Fas; (iii) the activation of EGFR is essential for the subsequent activation of ERK and Akt; and (iv) apoptotic keratinocytes secrete soluble EGFR ligands [50]. Pretel and colleagues, have found for the first time an increased expression of activated HER receptor isoforms in the basal layer of pemphigus mouse model skin lesions, confirming the involvement of EGFR in pemphigus. Besides, they observed higher level of activated mTOR within the basal cells of the epidermis [19]. After phosphorylation of HER receptor isoforms, intracellular signalling pathways are activated in the basal layer. In addition, the imbalance in Akt/mTOR that takes place in the basal cells may provide intracellular signals necessary for the development of apoptosis and acantholysis.

Several studies have clearly confirmed the critical role of apoptosis in pemphigus. Li and co-workers provided evidences on the relevance of the apoptotic mechanism in pemphigus foliaceous pathogenesis [25]. TUNEL-positive epidermal cells and increased oligonucleosomes in the epidermal cytosolic fractions were detected in PFIgG-treated mice. Timecourse study reveals that TUNEL-positive epidermal cells appear before intraepidermal blisters. Moreover, the proapoptotic factor Bax was up-regulated at earlier timepoints post PFIgG injection, whereas the antiapoptotic factor Bcl-x(L) was down-regulated at later timepoints, in association with the activation of executioner caspases. Administration of caspase-3/7 inhibitor protected mice from developing intraepidermal blisters and clinical disease induced by PFIgG. Collectively, these findings show that biochemical events of apoptosis are provoked in the epidermis of mice injected with PF autoantibodies. Caspase activation may thus contribute to blister formation in PF [25]. The observation that the cleaved caspase-3 and -6 are detected at late timepoints indicates that activation of these executioner caspases is a downstream event proximal to the onset of histological blistering

of PF. Possible upstream apoptotic pathways may include the mitochondrial as well as the death receptor pathway. Indeed, activation of Fas, FasL and caspase-8 has been shown in PVIgG-treated or sera-treated keratinocytes [23; 27; 18].

In addition, p38MAPK and heat-shock protein 70 (Hsp70) are activated in response to pathogenic PVIgG [16] and PFIgG [51]. This leads to reorganization of the actin cytoskeleton and to collapse of intermediate filaments in IgG-treated keratinocytes [16]. Inhibition of p38MAPK blocked pemphigus IgG-induced cytoskeletal reorganization in tissue culture and blistering in pemphigus mouse models. Subsequently, Lee and colleagues demonstrated two peaks of p38MAPK activation in pemphigus tissue culture and mouse models that lead to apoptosis. Administration of the p38MAPK inhibitor SB202190 before PFIgG injection blocked both peaks of p38MAPK phosphorylation and blister formation, and showed that apoptosis occurs at or after the second peak of p38MAPK activation [52]. It is interesting to note that Fas signaling leads to apoptosis also through the p38MAPK step [53], which may provide a mechanism for engagement of this stress kinase in the acantholytic pathway. In particular, binding of FasL to Fas rapidly activates p38MAPK, which in turn phosphorylates Bcl-xL and Bcl-2 and prevents the accumulation of these anti-apoptotic molecules within the mitochondria. Consequently, a loss of mitochondrial membrane potential and the release of cytochrome c lead to the activation of caspase-9 and, subsequently, caspase-3. Therefore, the activation of p38MAPK is a critical link between Fas and the mitochondrial death pathway. Moreover, cell detachment/mechanical stress *per se* can trigger p38MAPK, leading to overexpression of FasL [54], and induction of Hsp27 and Hsp70 [55]. Overexpression of active MAPK kinase, the activator of p38 MAPK, leads to activation of FasL promoter and induction of FasL transcripts. These results suggest that p38 MAPK is essential for FasL expression [56].

CONCLUSION

In substance, apoptosis exerts a pivotal role and precedes acantholysis in pemphigus. Therefore, apoptosis could represent a new target for pemphigus treatment. In particular, blister formation could be prevented by blocking specific apoptotic molecules, such as caspases, mTOR, p38MAPK [5] and FasL.

List of abbreviations:

PV, pemphigus vulgaris; PF, pemphigus foliaceus; dsg, desmoglein; PVIgG, pathogenic autoantibodies in pemphigus serum; p38MAPK, p38 mitogen-activated protein kinase; EGFR, epidermal growth factor receptor; FasL, Fas Ligand; TNF, tumor necrosis factor; DD, death domains; DED, death effector domains; DISC, Death-Inducing Signaling Complex; Hsp, heat-shock protein.

Conflict of interest:

Authors declare no conflict of interest.

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