Abstract

Pemphigus Vulgaris (PV) is a potentially fatal autoimmune blistering disease characterised by cell-cell detachment or acantholysis. The mechanisms which follow antibody (Ab) binding and culminate in acantholytic changes and skin/mucosal blistering have not been fully clarified. Current treatment strategies are not specific to PV pathophysiology and although life-saving, harbour considerable side effects. We aimed to systematically assess the molecules amenable to targeted treatments that follow Ab binding and are associated with PV acantholysis. The resulting scoping review was conducted under PRISMA- ScR guidelines with clear inclusion and exclusion criteria and focused specifically on kinases, caspases, proteases, hydrolytic enzymes and other molecules of interest postulated to take part in the pathophysiology of PV. The review process resulted in the identification of 882 articles, of which 56 were eligible for qualitative synthesis. From the included articles, the majority (n=42) used PV-IgG as the pathogenic agent, mainly via in vitro (n=16) and in vivo (n= 10)
models. Twenty-five molecules were found to play a pathogenic role in PV, including uPA, ADAM10, EGFR, Src, PKC, cdk2, ERK, PLC, nNOS, calmodulin, NOS, p38MAPK and caspase-3. Selective inhibition of these molecules resulted in varying degrees of reduction in acantholysis and blistering. The pathogenic molecules identified in this review represent potential candidates for clinical translation.

Keywords: pemphigus vulgaris, acantholysis, kinases, caspases, molecular pathways

Abbreviations
ADAM10 metalloproteinase 10 □ ASK1 apoptosis signal-regulating kinase □ BTK bruton tyrosine kinase □ CDK cyclin dependent kinase □ CRAC calcium release activated calcium channel protein 1 □ DAG diacylglycerol □ DSG desmoglein □ EDTA ethylene diamine tetraacetate □ EGF epidermal growth factor □ EGFR epidermal growth factor receptor □ ERK extracellular signal-regulated kinase □ FAK focal adhesion kinase □ IP3 inositol trisphosphate □ IP3R inositol 1,4,5 trisphosphate receptor □ NEM N.ethylmaleimide □ nNOS neuronal nitric oxide synthase □ NO nitric oxide □ NOS nitric oxide synthase □ P38 MAPK p38 mitogen-activated protein kinase □ PA plasminogen activator □ PKC phosphokinase C □ PKP3 plakophilin 3 □ PLC phospholipase C □ ROS reactive oxygen species □ SCR sarcoma associated kinase □ SBTI soybean trypsin inhibitor □ Trx2 thioredoxin-2 □ uPa urokinase plasminogen activator □ uPar urokinase plasminogen activator receptor □ YAP yes-associated protein □

Introduction
Pemphigus Vulgaris (PV) is an autoimmune blistering condition affecting 0.1 to 0.5 per 100,000 people worldwide\(^1\). Before the advent of corticosteroids, PV was invariably fatal and even when treated, mortality rates are as high as 15%\(^2\). PV is characterised by painful flaccid
bullae with mucosal or mucocutaneous distribution and commonly manifests orally\textsuperscript{3, 4}. Histologically, intraepithelial splitting of the suprabasal epidermal layer is visible. The disease process is triggered by a diverse array of autoantibodies (PVIgG), including those directed against Desmoglein (Dsg) 1 and Dsg3 cadherin family molecules, responsible for the cohesion of keratinocytes\textsuperscript{5-7}. Disruption of cell-cell adhesion, which ultimately drives the pathological process in PV, is the result of the activation of complex intracellular cascades in response to PVIgG.

Despite the scientific progress surrounding PV pathogenesis, treatment options remain limited to immunosuppression\textsuperscript{2, 8}. First line therapy consists of high-dose corticosteroids with use of additional nonsteroidal immunosuppressive agents\textsuperscript{9}. Unfortunately, these medications have significant side effects which may be aggravated and compounded in complexity with long-term use\textsuperscript{10, 11}. Further research into newer treatment modalities is therefore necessary\textsuperscript{12}. Crucially, elucidation of the pathogenic pathways of PV-induced acantholysis may inform the selection of specific drugs and pathway modifiers that can be used to prevent blister formation.

The exact molecular mechanisms of PV and acantholysis are still under investigation. It was originally proposed that anti-Dsg autoantibodies were necessary and sufficient to cause the disease. This monopathogenic theory suggests binding of anti-Dsg autoantibodies drives steric hindrance, keratinocyte dissociation and acantholysis\textsuperscript{13}. Furthermore, PVIgG specificity is not limited to Dsg as these autoantibodies target other molecules including mitochondrial antigens and Ach receptors and act synergistically with anti-Dsg autoantibodies\textsuperscript{14-16}. In addition to steric hindrance, it is now accepted that binding of PVIgG to keratinocytes alters signalling of various intracellular molecules, including p38 mitogen-activated protein kinase (p38MAPK), Phosphokinase C (PKC), Rho A and Caspase 3, 6, 8 and 9\textsuperscript{17}. The activation of caspase has thus coined the term “apoptolysis”, suggesting a common link between apoptosis and acantholysis, further contributing to the intracellular evidence behind the disease\textsuperscript{18}. Despite the plethora of intracellular pathways taking part in acantholysis, the pathogenicity of the molecules purportedly involved in PV signalling has not yet been fully elucidated.
The aim of this scoping review was to investigate the pathogenic role of intracellular signalling molecules in the pathogenesis of PV.

**Methods**

*Search Strategy*

The protocol for this review followed the Preferred System for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR).

An extensive search of the MEDLINE/Pubmed and medRxiv databases was performed from April to July 2020 and subsequently updated in July 2021. The term pemphigus vulgaris was searched in conjunction with the following five key categories and associated terms: 1. caspases, 2. proteases, 3. kinases and phosphatases, 4. hydrolytic enzymes, and 5. other molecules. These terms were combined with the use of Boolean operators, computed through the database and analysed (Suppl Table 1). The final search was performed by five key investigators.

*Eligibility Criteria*

Articles were included in this review if they met the following criteria: (a) described and (b) demonstrated the pathogenic role of the molecule(s) under investigation. The pathogenicity criterion was satisfied if the study met the following: (i) PV serum, PV-IgG, PV-specific monoclonal antibodies or non-IgG serum factors from PV patients induced PV-like phenotype *in vivo* or acantholysis/intercellular detachment *in vitro*; or study in humans (PV patient); and (ii) a specific pharmacological block, knock-out, knock-down, silencing or inactivation, knock-in or activation, of downstream signalling molecule prevented or significantly reduced PV phenotype *in vivo*, acantholysis/intercellular detachment *in vitro*, or ameliorated blistering/clinical manifestations in PV patients. Exclusion criteria were the following: (a) non-English (b) review (c) forms of pemphigus other than PV. There was no time restriction.

*Data Collection and Quality Assessment*

The selection process for each search was performed independently by two reviewers. The initial phase of the search process involved excluding reviews and non-English articles. In the
second phase, titles and abstracts were screened according to their relevance to “pemphigus vulgaris,” and duplicates from the individual searches were removed. In the final phase, full text was screened in accordance to the specific inclusion criteria and selected accordingly. Information from selected articles was then gathered and tabulated. At the end of each phase, the two reviewers provided independently a final judgement (include, exclude or uncertain) of inclusion for the selected articles and notified such recommendations to the senior author (NC). In cases of disagreement the same author took a final decision of the inclusion after discussion with the first two reviewers in a joint meeting.

Results

The five key categories were investigated individually and then combined to produce a total of 882 articles identified following the initial search strategy (Suppl Table 1). Figure 1 highlights the selection process that was implemented, leaving 159 articles to be screened against the inclusion criteria. As a result, 56 articles were included in the final review (Table 1). From the included papers, majority (n=42) used PV-IgG as the pathogenic agent, via in vitro (n=16) methods, in vivo (n=10) models, or a combination of both (n=7). PV-IgG was also used in ex vivo models (n=4), a combination of in vitro and ex vivo (n=2), a combination of in vivo and ex vivo (n=1), as well in all 3, in vitro, in vivo and ex vivo (n=1). PV patients were also studied (n=1) (Table 2). In general, inhibition of target molecules, including caspases, proteases, kinases, hydrolytic enzymes, and other molecules such as NOS, calmodulin or calcium, resulted in a decrease in acantholysis and reduction in the PV-like phenotype.

1. Caspases

A total of seven studies demonstrated the pathogenicity of caspases in PV. Two studies used in vitro models where PV-IgG was administered to in vitro human keratinocyte and mouse keratinocyte cultures, one used in vitro human keratinocytes and skin cultures, one used in vitro human keratinocyte cultures only and three were conducted in vivo using a mice model. Caspase inhibitors Ac-DEVD-CMK\textsuperscript{19}, cpm-VAD-CHO\textsuperscript{20, 21}, ZDCB-MK\textsuperscript{22}, caspase inhibitor
III \(^{23}\) and Z-IETD-FMK \(^{24}\) were used in the studies. The common finding within all studies was that caspase inhibition led to a decrease in PV induced apoptosis and acantholysis.

2. **Proteases**

A total of 16 studies demonstrated a pathogenic role of proteases in PV. 8 studies used in vitro models where PV-IgG or PV-Serum was administered to either human keratinocyte cultures, mouse keratinocyte cultures, 4 studies used \textit{ex vivo} models and one used in combination of both \textit{in vitro} and \textit{ex vivo}. One study also used in vivo mouse models and two used a combination of \textit{in vivo} c57BL or BALB/c mice passive transfer models as well as cell cultures. Overall, the inhibition of proteases resulted in a reduced incidence or complete inhibition of acantholysis. Pathogenic proteases were the serine proteases within the uPA system and ADAM10\(^{23-38}\).

3. **Kinases and Phosphatases**

A total of 29 studies demonstrated pathogenicity of kinases in PV. Fourteen of the studies used \textit{in vitro} models where PV-IgG or PV serum was administered to HaCaT cells or primary keratinocytes. Four studies used \textit{in vivo} passive mouse models injected with AK23 or PV-IgG, and eight studies used a combination of both. One study also used \textit{ex vivo} human skin models with PV-IgG. One paper used all three, \textit{in vivo}, \textit{in vitro} and \textit{ex vivo} models. The remaining study used patients diagnosed with PV. Kinases p38MAPK, sarcoma associated kinase (Src), extracellular signal-regulated kinase (ERK), PKC, bruton tyrosine kinase (BTK), apoptosis signal-regulating kinase (ASK1), epidermal growth factor receptor kinase (EFGRK), tyrosine kinase (TK), mitogen-activated protein kinase (MAPK)-activated protein kinase 2 (MK2) and cyclin dependent kinase 2 (CDK2), were found to be phosphorylated in PV-induced models\(^{16, 39-65}\). Inhibition of kinase signalling reduced or completely prevented acantholysis \textit{in vitro} and blister formation \textit{in vivo}.

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4. Hydrolytic Enzymes

A total of four studies demonstrated pathogenicity of hydrolytic enzymes in PV. All studies except one looked specifically at phospholipase C (PLC) inhibition using PLC inhibitor U73122. The use of inhibitor U73122 was used in one in vitro, one ex vivo and one in vivo mouse model study. These studies resulted in the inhibition of acantholysis via PLC inhibition, which in turn contributed to the blocking of transmembrane signalling involved in cell-to-cell detachment by PV-IgG binding. However, one study reported that the addition of PI-PLC in the culture medium reduced acantholysis via uPAR cleavage.

5. Other Molecules

A total of 10 studies demonstrated pathogenicity of molecules not included in the categories above in PV. One study focused on intracellular calcium and calmodulin, two on Nitric Oxide Synthase (NOS), one on mitochondrial targets, one on NOD2, one of FasL, one on muscarinic pathways, one on IP3R and CRAC, one on RhoA and one on HER. Subjects utilised included in vivo mouse models (n=5), ex vivo models (n=3), in vitro human keratinocytes (n=2) and a combination of in vivo and in vitro (n=1). Seven studies utilised PV-IgG as the pathogenic agent whereas two studies used PV patient serum.

The overall trend demonstrates that inhibition of these targets resulted in the amelioration of acantholysis. Nevertheless, the interplay between these molecules is complex requiring further research.

Discussion

This scoping review evaluates the literature pertaining to the pathogenic intracellular mechanisms observed in PV. Overall, we individuated 25 pathogenic molecules (Table 1). Six molecules in vivo, three in vitro, and ten in both in vivo and in vitro. Five molecules were also identified in both in vitro and ex vivo (Table 2). Targeting of these pathogenic molecules had not been successfully translated into PV patient treatment until a BTK was recently tested in a...
phase II trial. On the basis of our results, the pathogenic molecules and pathways amenable for mechanism-based treatments of PV are discussed in the following paragraphs.

1. Caspases

Caspases play an important role in the pathogenesis of PV, through apoptosis and acantholysis. Caspases are activated via intrinsic and extrinsic cellular pathways to carry out programmed cell death. While apoptosis through caspases is a normal process in the body, PV-IgG also causes pathological activation of caspases in keratinocytes. All studies examined reveal that inhibiting caspases causes a decrease in apoptosis and acantholysis, leading to a positive effect on cell-to-cell adhesion. There may be a relationship between apoptosis and acantholysis itself; Pretel, et al. report that as apoptosis appears before acantholysis, inhibiting the former through caspase inhibitors will inhibit acantholysis. This requires further research as other studies such as Schmidt, et al. assert that apoptosis is not a prerequisite for acantholysis. The selected studies in this review suggest inhibition of caspases causing decrease in apoptosis, assuming that acantholysis would also decline. With this in mind, one is forced to contemplate whether caspase inhibition would have a significant therapeutic benefit for PV if acantholysis is not inhibited. Nonetheless, these findings do not coincide with those of others discussed in this review and further research is warranted for definitive results.

Majority of studies focus on caspase-3 which is a prominent effector in keratinocytes. Caspase-3 inhibition through naringenin appears to have a positive effect on cell-to-cell contact. Pacheco-Tovar, et al. provide further support to the idea that caspases play a key role in apoptosis and blister formation.

Consideration of other upstream and downstream cell signalling pathways is essential. Gil, et al. delve into the relationship between caspases and focal adhesion kinase (FAK). They reveal that inhibiting caspases reduces FAK activation, hence markers of PV lesions are completely absent.
Overall, most studies have shown similar results, hence inhibiting caspases is a potential mechanism to alleviate PV symptoms. Further research is required to determine the exact role of caspases in apoptosis/acantholysis and how caspases may interact with other intracellular molecules.

2. Other proteases

Proteases are enzymes which hydrolyse the peptide bonds within proteins. Early studies on the pathogenesis of PV have implicated proteases through use of broad-spectrum protease inhibitors such as N-ethylmaleimide (NEM), ethylene-diamine-tetraacetate (EDTA) and soybean trypsin inhibitor (SBTI) to inhibit PV-associated acantholysis. More recent research has looked at 2 key proteases: metalloproteases and serine proteases.

Serine Proteases and the Plasminogen Activator System

Urokinase plasminogen activator (uPA) is a serine protease which catalyses the conversion of plasminogen to plasmin. Multiple studies have shown the upregulation of expression and activity of uPA and its receptor (uPAR) following PV-IgG administration. Additionally, both in vitro (e.g. using human/mouse keratinocyte cultures and human skin explants) and in vivo (e.g. neonatal BALB/mice) studies have demonstrated that inhibition of either uPA or uPAR can lead to partial or complete inhibition of PV-mediated acantholysis. However, the involvement of uPA/uPAR in the pathogenesis of PV currently holds less credit due to two major findings. Some studies have suggested that the PV-IgG associated upregulation of uPA/uPAR expression and activity might be involved in wound re-epithelialisation following acantholysis rather than preceding it. Secondly, Mahoney et al. found that uPA knockout mice blistered to the same degree as control mice, demonstrating that the plasminogen activator system is not necessary for PV-IgG to induce acantholysis in PV mouse models.

Metalloproteases

A disintegrin and metalloproteinase 10 (ADAM10) induction via an Src-dependent pathway, coupled to an increase in epidermal growth factor (EGFR) ligands and beta-cellulin has been
implicated in the acantholytic process in patients whose PV-IgG contain anti-Dsg1/3\(^3\)\(^4\). The inhibition of this pathway at any stage (ADAM10, EGF, EGFR or Src) has been shown to reduce or ameliorate supra-basal acantholysis in neonatal mice. Interestingly, PV-IgG serum containing anti-Dsc2/3 antibodies seemed to trigger more severe acantholysis in a shorter amount of time, via an ADAM10-independent pathway\(^3\)\(^4\). In samples containing both anti-Dsc2/3 and anti-Dsg3 antibodies, the severity of acantholysis was shown to be dependent on the level of anti-Dsc2/3, with decreasing levels of anti-Dsc2/3 resulting in reduced acantholytic severity, even when antiDsg3 levels were held constant\(^3\)\(^4\). ADAM10 inhibitors in these samples were not able to deter cell-cell detachment. These findings give credit to the ‘multi-pathogenic theory’ which postulates that suprabasal acantholysis occurs via the ‘multiple hit hypothesis’\(^8\)\(^1\); and the range of phenotypic variations occur due to differing autoantibody profiles across patients. While ADAM10 and the Src-dependent pathway may be a mechanism-based therapeutic target for a subtype of PV-IgG profiles, several other signalling pathways downstream of Dsg/non-dsg antibody binding may still induce PV-associated acantholysis.

3. Kinases and Phosphatases

Intracellular kinase signalling has been identified in the pathogenic process of PV. Attachment of autoantibodies in PV promotes phosphorylation of kinases (PKC, p38MAPK, Scr, ERK)\(^4\)\(^2\), \(^4\)\(^5\). Inhibition of these pathways reduces acantholysis in vitro and in vivo.

The role of p38MAPK has been highlighted in multiple studies. p38MAPK is activated after PV-IgG binding upstream of Rho kinase. Studies show an increase in phosphorylation after PV-IgG treatment\(^4\)\(^0\), \(^4\)\(7\). Inhibition using SB202190 prevents internalisation of the PV-IgG Dsg-3 complex and protects against endocytosis and acantholysis\(^1\)\(^6\), \(^4\)\(4\), \(^4\)\(5\), \(^4\)\(7\), \(^5\)\(0\), \(^5\)\(1\), \(^5\)\(8\), \(^6\)\(1\), \(^8\)\(2\). In particular, p38MAPK inhibition prevents PV-IgG induced redistribution of Dsg3 and results in PV-IgG and Dsg3 co-localising at the cell membrane\(^4\)\(^0\), \(^5\)\(0\). As a result, p38MAPK inhibition has been shown to prevent both histologic and clinical blister formation in both in vivo and in

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vitro models. Therefore, p38MAPK plays an essential role in regulating Dsg3 internalisation and must be considered as a key component in PV pathogenicity.

Furthermore, Src is also activated by PV-IgG and AK23 early on. In PV patients, Src triggers the phosphorylation of plakophilin 3 (Pkp3), along with Dsg3 dissociation and loss of cell adhesion. PP2 is commonly used to block the effects of Src in PV induced models. Inhibition of Src reduces PV-IgG and Ak23 induced loss of cell adhesion. In addition, blocking Src activity blocks Pkp3 phosphorylation and its detachment from Dsg3, resulting in a decrease in acantholysis. Inhibition of Src may be patient dependent, as it prevents Ak23 induced skin blistering in mice models but not in all ex vivo human epidermis models. In particular, activation of Src occurs when PV-IgG fractions have antibodies acting against Dsg3. Since AK23 affects Dsg3 specifically, Dsg3 is able to regulate Src signalling. However, Src inhibition may not be sufficient alone in PV pathogenesis and should be considered in relation with other kinases in treatment.

Other notable kinases involved in PV pathogenesis are ERK and PKC. ERK activation occurs in mc-PV-IgG and is dependent on EGFR and Src. ST18 overexpression alters ERK activity, inducing significant ERK activation after AK23 or PV-IgG treatment. ERK inhibitor U0126 is able to prevent loss of cell adhesion. PKC, a downstream signalling enzyme to PLC is also activated in PV-IgG cultured cells and mice. It is activated by intracellular calcium and directly modifies desmosome adhesive states. Inhibition of PKC, using Bim-X or Go6976, reduces rate of acantholysis, processing of cell adhesion molecules such as cadherin and their depletion from cell surfaces is reduced.

Recent research has also shown the Trx2/ASK1 signalling pathway is activated in PV. This signalling pathway plays a key role in regulation of mitochondrial damage. In the presence of PV serum, Trx2 expression decreases and phosphorylation of ASK1 increases. However, when Trx2 is silenced, there is a reduction of apoptosis of HaCaT cells that are treated with PV serum. In the presence of GS-44217 which is an ASK1 specific inhibitor, apoptosis is reduced.
also decreased. In vivo, mice that were treated with overexpression of Trx2 in the presence of PV serum demonstrated a reduction in acantholysis.

In addition, Burmester et al., recently observed the effects of inhibiting MEK1, TrkA, P13Kα, and VEGFR2 in vitro using human keratinocytes and ex vivo using human epidermis. A comparison was made the to the p38 MAPK inhibition pathway studied by Sayer et al., 2014 in which patients treated with the p38 MAPK inhibitors showed no clinical benefit. By targeting MEK1, TrkA, P13Ka, VEGFR2 and their downstream targets, anti-Dsg1/3-induced pathology was impaired and therefore intraepidermal blistering was altered.

Of the new potential therapies for pemphigus, the BTK inhibitor rilzabrutinib (PRN1088) has recently been tested in patients with PV in a phase II trial. Patients diagnosed with PV were administered oral rilzabrutinib for 12 weeks with or without corticosteroids. Out of the 27 patients, 14 patients had no new lesions and showed control of disease activity within 4 weeks. However, 11 of those patients were also receiving low dose of corticosteroids while 3 were not. Nevertheless, the rate of control of disease activity was between 50-56% for moderate to severe PV. Therefore, the inhibition of kinases such as BTK demonstrate a promising potential for future treatment of PV.

4. Hydrolytic Enzymes

Hydrolytic enzymes play a role in the pathogenesis of PV by inducing intracellular signalling transduction. The role of hydrolytic enzymes in signal transduction occurs after extracellular binding of PV-IgG. Specifically, PLC hydrolyses phosphatidylinositol 4,5-bisphosphonate leading to the production of IP3, calcium production, and ultimately acantholysis. By inhibition of PLC via U73122 PLC inhibitor, IP3 production, calcium release, and inhibition of PV-IgG induced detachment of cell-cell contacts occurs. PLC inhibition also shows the reduced secretion of PA and thus inhibition of acantholysis. Both human sera and in vivo mice models with the use of PLC inhibitor U73122 highlighted the role PLC has on the intracellular pathways leading to acantholysis of PV. Sanchez-Carpitero et al. found that
injection of PLC inhibitors into mice prior to injection of PV-IgG resulted in prevention of PV-IgG-induced blistering in vivo\(^6\). Furthermore, PLC and IP3R inhibitors have demonstrated decreased PV IgG induced calcium influx. Schmitt et al highlighted that inhibition of these molecules was protective against ex vivo PV IgG induced blistering\(^7\). These studies further support the use of intracellular signalling cascades as a target for PV treatment in humans.

5. Other molecules

The pathogenicity of PV is complex and there are numerous other key intracellular molecules involved. Intracellular calcium is paramount to intracellular signalling and enzyme activation. The binding of PV-IgG to keratinocyte antigens causes intracellular calcium levels to increase\(^16,8\). Seishima et al demonstrated that extracellular binding of PV-IgG drives hydrolysis of phosphatidylinositol 4,5-bisphosphonate through PLC activation. This drives IP3 production, releasing calcium from intracellular stores activating Protein Kinase C eventually driving acantholysis and the clinical signs of PV\(^8\). Sanchez-Carpintero et al highlighted the importance of calcium as a driving force of acantholysis. Cells treated with calmodulin inhibitors (W7 Hydrochloride) resulted in disease free histological sections and the absence of acantholysis\(^6\). Upstream, it has been noted that cholinergic activation may also play a role in acantholysis. Chernyavsky et al highlighted a decrease in acantholysis through P120-catenin inhibition when cells were exposed to Alpha-7 nicotine agonists in the presence of PV-IgG\(^7\). Similarly, FasL and intracellular calcium share common signalling pathways. Administration of anti-FasL Abs following PV-IgG exposure has shown to decrease acantholysis in keratinocytes\(^7\).

Recently, great focus has been attributed to oxidative stress and its pathogenic link in PV. Mitochondria therefore play a key role in this connection\(^1\). Immunoblotting assays of mouse keratinocytes have highlighted PV-IgG binding to mitochondrial antigens. This results in increased cytochrome c release, disruption of inner membrane of the mitochondrial electron transport chain, decreased ATP production and consequent increase in reactive oxygen species (ROS) production\(^15\). Ultimately, damage to the mitochondrial power chain triggers apoptosis.
The administration of mitochondrial protecting medications (minocycline, nicotinamide, cyclosporine) can eradicate acantholysis in a PV-IgG induced mouse model. The clinical significance of oxidative stress induced acantholysis is further highlighted by Liang et al, demonstrating decreased cell-cell detachment through naringenin induced inhibition of NOD2 and NF-κB pathways. Other non-Dsg molecules include Nitric Oxide (NO) Synthase. These are not necessarily targeted by PV-IgG however increased levels are highlighted in acantholytic cells suggesting they play a key role in molecular signalling. It is important to note that immunostaining of disease-free individuals was negative for NOS. España et al demonstrated the pathological importance of nNOS, highlighting a decrease in acantholysis in cells pretreated with nNOS inhibitors. Of note, different types of NOS exist and do not share similar results.

The role of YAP in PV has also been explored recently. Huang et al (2021) found an increase in YAP protein levels in cells treated with PV serum. On knock down of YAP using sRNA, silencing resulted in elimination AK23 mediated Dsg3 depletion. In particular, when YAP is silenced, there is a significant two-to-three-fold increase in Dsg3 expression in the presence of AK23.

**Limitations**

It should be noted that most studies were performed in animal models or in vitro. While experimental inhibition of these molecules resulted in histological or phenotypic improvements in PV-associated acantholysis, it is unclear how interference in such pathways would impact on normal human physiology. Hence, the feasibility of targeting these molecules in humans might be limited by potential side-effects of inhibiting essential molecules. Additionally, pre-determined search categories addressing specific molecular classes were used, and this could have resulted in overlooking a number of key molecules not belonging to these molecular pathways. For example, the review does not include the pathogenic molecules upstream of PV IgG action (e.g. PV antigens). Also, researchers in this review examined these categories of intracellular molecules independently. Therefore, from this scoping review it may be difficult to ascertain the precise interplay between various intracellular molecules in...
PV pathogenesis and how each molecule fits into the multi-pathogenic theory that has been put forward.

This review assessed findings from 56 papers that met the inclusion criteria, however, other relevant papers may have been overlooked due to limitations relating to the selected search terms. For example, on manual search, a recent paper by Burmester et al. found that the inhibition of MEK1, TrkA, P13Kα, VEGFR2 impaired PV IgG prevented intraepidermal split formation in human skin cultures.85

Conclusion
This review has highlighted the complex molecular mechanisms that drive the pathogenicity of PV. In addition to the pathogenic Dsg antibodies, there are other molecules and intracellular signalling pathways at play. The importance of some of these intracellular molecules as well as inhibition of themselves or their activating counterparts have been noted to inhibit acantholysis and have been emphasised in this review. With further research into these pathways, this may provide scope for the development of newer and better therapeutic agents for PV treatment.

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Author contributions
NC conceived the idea and rationale for a scoping review, designed the study and search strategy, and supervised the study selection process. BK, JK, JK, MK, SK performed all the steps of the systematic search, collected and analyzed all of the data, and wrote the first draft as well as revised versions of the manuscript under the guidance of the senior author (NC). NC critically revised the manuscript.
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Table 1. Pathogenic signalling mechanisms in PV – caspases and apoptosis, proteinases, kinases, hydrolytic enzymes and other molecules.

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<td>Caspase-3 and proteases like calpain targeted by Pan-Caspase inhibitor</td>
<td>Mouse keratinocyte cultures Mouse keratinocyte cultures</td>
<td>Keratinocyte-specific caspase-3 deficient mice (casp3EKO) injected with pathogenic Dsg3 antibody (AK23) had less severe oral blistering. Injection of neonatal mice with caspase-3 inhibitor III reduced PF/AK23 IgG (antiDsg1/3)-induced epidermal lesions by 60%. Pre-treatment with pan-caspase and two caspase-3 inhibitors led to a significant reduction in AK23-mediated loss of intercellular adhesion in mouse and human keratinocyte cultures.</td>
</tr>
<tr>
<td>Wang et al.</td>
<td>2004</td>
<td>Apoptosis</td>
<td>PV-IgG</td>
<td>Proteases Caspases</td>
<td>Broad spectrum inhibition</td>
<td>Human keratinocyte cultures Human skin ex vivo</td>
<td>Incubation with caspase inhibitor prevents keratinocyte death and tissue acantholysis induced by PV-IgG.</td>
</tr>
<tr>
<td>Feliciani et al.</td>
<td>2003</td>
<td>Experimental Dermatology</td>
<td>PV Serum</td>
<td>Proteases</td>
<td>uPA</td>
<td>Human keratinocyte cultures</td>
<td>Pre and co treatment with anti-uPA resulted in 90% suppression of acantholysis</td>
</tr>
<tr>
<td>Puviani et al.</td>
<td>2003</td>
<td>The Journal of Investigative Dermatology</td>
<td>PV Serum</td>
<td>Proteases Caspases</td>
<td>FasL Caspase-8</td>
<td>Human keratinocyte cultures</td>
<td>Pre-treatment with Anti-FasL neutralizing antibody, and caspase-8 inhibitor (Z-IETD-FMK) separately, partially inhibited PV-sera-induced keratinocyte apoptosis</td>
</tr>
<tr>
<td>Asano et al.</td>
<td>2001</td>
<td>Experimental Dermatology</td>
<td>PV Serum</td>
<td>Proteases Hydrolytic enzymes</td>
<td>uPA PI-PLC</td>
<td>DJM-1 Squamous carcinoma cell line</td>
<td>Pre-incubation with PI-PLC inhibited cell-cell detachment at 10g/mL Pre-incubation with tranexamic acid inhibited cell-cell detachment at 15mM.</td>
</tr>
<tr>
<td>Xue et al.</td>
<td>1998</td>
<td>The Journal of Cutaneous Pathology</td>
<td>PV-IgG</td>
<td>Proteases</td>
<td>uPAR</td>
<td>Human keratinocyte cultures</td>
<td>Incubation with anti-uPA-R IgG (100g/ml) significantly inhibited pemphigus IgG induced acantholysis</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Year</td>
<td>Journal</td>
<td>PV-IgG</td>
<td>Proteases</td>
<td>uPA</td>
<td>Skin model</td>
<td>In-vitro Incubation</td>
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<tr>
<td>Dobrev et al.</td>
<td>1996</td>
<td>Archives of Dermatological Research</td>
<td>PV Serum</td>
<td>Proteases</td>
<td>Plasminogen Activators and Trypsin</td>
<td>Human skin ex vivo</td>
<td>In-vitro incubation with 1mg/ml PAMBA could completely inhibit PV-IgG induced acantholysis. Incubation with 10 ATE/ml aprotinin could partially inhibit PV-IgG-induced acantholysis.</td>
</tr>
<tr>
<td>Naito et al.</td>
<td>1989</td>
<td>The Journal of Investigative Dermatology</td>
<td>PV-IgG</td>
<td>Proteases</td>
<td>Broad spectrum protease inhibition</td>
<td>Human skin ex vivo Mouse model</td>
<td>In vitro: FOY (1-10mg/ml), FOY-305 (0.55mg/ml) and FUT-175 (0.1-5mg/ml) were able to inhibit acantholysis in skin culture at all concentrations tested. Three or 12hr pre-incubation with alpha-1PI inhibited acantholysis at 25mg/ml but not at 2.5mg/ml. In vivo: Only pre-injection of Alpha-1-PI (2.5mg) - but not FOY, FOY-305 or FUT-175 - could inhibit both the Nikolsky sign and acantholysis completely in mice.</td>
</tr>
<tr>
<td>Wilkinson et al.</td>
<td>1989</td>
<td>American Journal of Pathology</td>
<td>PV-IgG</td>
<td>Proteases</td>
<td>uPA</td>
<td>Canine oral keratinocyte cultures</td>
<td>Treatment of cell cultures with human placental uPA inhibitor resulted in a marked reduction in number of keratinocyte cell cultures with acantholytic lesions.</td>
</tr>
<tr>
<td>Morioka et al.</td>
<td>1987</td>
<td>The Journal of Investigative Dermatology</td>
<td>PV-IgG</td>
<td>Proteases</td>
<td>uPA</td>
<td>Human skin ex vivo</td>
<td>Skin explants cultured with anti-urokinase IgG completely inhibited PV IgG induced acantholysis.</td>
</tr>
<tr>
<td>Woo et al.</td>
<td>1983</td>
<td>The Journal of Investigative Dermatology</td>
<td>PV-IgG</td>
<td>Proteases</td>
<td>Broad spectrum protease inhibition, including uPA.</td>
<td>Neonatal BALB/c primary epidermal cell cultures</td>
<td>Treatment with broad spectrum proteinase inhibitors inhibited PV-IgG induced cell detachment. SBTI produced 62%, aprotinin 63%, 2M 70%, pepstatin 49% and LBTI 54% inhibition of the PV-IgG induced cell detachment.</td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>Journal</td>
<td>PV-IgG</td>
<td>Proteases</td>
<td>Plasmin</td>
<td>Human skin ex vivo</td>
<td>Summary</td>
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<tr>
<td>Hashimoto et al.</td>
<td>1983</td>
<td>The Journal of Experimental Medicine</td>
<td>PV-IgG</td>
<td>Proteases</td>
<td>Plasmin</td>
<td>Human skin ex vivo</td>
<td>Both Aprotinin and LBTI, when used separately, were able to inhibit PV-IgG induced acantholysis in skin explants.</td>
</tr>
<tr>
<td>Morioka et al.</td>
<td>1981</td>
<td>The Journal of Investigative Dermatology</td>
<td>PV-IgG</td>
<td>Proteases</td>
<td>Plasmin</td>
<td>Human skin ex vivo</td>
<td>PV-IgG induced acantholysis was completely inhibited by incubation with 2000g/ml of SBTI as well as 40g/m of pepstatin A.</td>
</tr>
<tr>
<td>Singer et al.</td>
<td>1980</td>
<td>The Journal of Investigative Dermatology</td>
<td>PV Serum</td>
<td>Proteases</td>
<td>Plasmin</td>
<td>Neonatal BALB/c primary epidermal cell cultures</td>
<td>Incubation of cell cultures with SBTI (100 g/ml) or 2M (0.5 mg/ ml) resulted in inhibition of PV serum induced cell detachment.</td>
</tr>
<tr>
<td>Farb et al.</td>
<td>1978</td>
<td>Proceedings of the National Academy of Sciences</td>
<td>PV-IgG and PV Serum</td>
<td>Proteases</td>
<td>Plasmin</td>
<td>Neonatal BALB/c primary epidermal cell cultures</td>
<td>Pre-treatment of cell cultures with SBTI (100g/ml) or 2M (0.5 mg/ ml) resulted in the inhibition of pemphigus-induced cell detachment.</td>
</tr>
<tr>
<td>Ivars et al.</td>
<td>2019</td>
<td>British Journal of Dermatology</td>
<td>PV-IgG</td>
<td>Proteases</td>
<td>Plasmin</td>
<td>Mouse model</td>
<td>Injection of Src inhibitor and EGFR inhibitors resulted in clinical detachment (Nikolsky sign) and histological acantholysis in C57BL/6J mice with PV1-IgG and PV2-IgG fractions, but not PV3-IgG.</td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>Journal</td>
<td>PV-IgG/anti-Dsg3 IgG/Ak23</td>
<td>Kinases</td>
<td>Kinase Inhibitor</td>
<td>Tissue Type</td>
<td>Treatment Details</td>
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<tr>
<td>Lee et al.</td>
<td>2009</td>
<td>The Journal of Biological Chemistry</td>
<td>PV-IgG</td>
<td>Kinases</td>
<td>P38MAPK</td>
<td>Human keratinocytes cultures Mouse model</td>
<td>Pre-treatment with p38MAPK inhibitor to inhibit the first peak, but not the second peak of p38MAPK activity resulted in inhibition of PV-IgG-induced cytokeratin retraction and acantholysis in human keratinocyte cultures.</td>
</tr>
<tr>
<td>Egu et al.</td>
<td>2019</td>
<td>Frontiers to Immunology</td>
<td>PV-IgG</td>
<td>Kinases</td>
<td>ERK PKC</td>
<td>Human skin ex vivo</td>
<td>Inhibition of ERK using U0126, reduced blister formation and prevented the decrease of desmosomes. Inhibition of PKC however did not prevent suprabasal blister formation.</td>
</tr>
<tr>
<td>Walter et al.</td>
<td>2019</td>
<td>Frontiers to Immunology</td>
<td>PV-IgG</td>
<td>Kinases</td>
<td>EGFR ERK Src</td>
<td>Human keratinocyte cultures</td>
<td>Inhibition of Src and ERK using PP2 and U0126, reduced the amount of cell adhesion loss in response to PV-IgG. EGFR and ERK activation was Src and EGFR kinase dependent.</td>
</tr>
<tr>
<td>Kugelmann et al.</td>
<td>2019</td>
<td>Frontiers to Immunology</td>
<td>PV-IgG Anti-Dsg3 IgG/Ak23</td>
<td>Kinases</td>
<td>Src</td>
<td>Human keratinocyte cultures</td>
<td>Inhibition of Src using PP2 significantly reduced PV-IgG and AK23 induced loss of cell cohesion in vitro and in vivo. Src inhibition did not affect PV-IgG induced skin blistering ex vivo.</td>
</tr>
<tr>
<td>Radeva et al.</td>
<td>2019</td>
<td>Frontiers to Immunology</td>
<td>PV-IgG Anti-Dsg3 IgG/Ak23</td>
<td>Kinases</td>
<td>ERK</td>
<td>Human keratinocyte cultures</td>
<td>Inhibition of ERK using U0126 completely abolished PV-IgG and AK23 autoantibody induced loss of cell adhesion, in ST18 transfected monolayers.</td>
</tr>
<tr>
<td>Rotzer et. al&lt;sup&gt;42&lt;/sup&gt;</td>
<td>2015</td>
<td>Cell Mol Life Sci</td>
<td>PV-IgG</td>
<td>Kinases</td>
<td>p38MAPK Src</td>
<td>Mouse model</td>
<td>Inactivation using Sb202190 and PP2 resulted in overexpression of Ecadherin-GFP, and blocked the dose-dependent activation of p38 MAPK induced by AK23.</td>
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<tr>
<td>Saito et. al&lt;sup&gt;45&lt;/sup&gt;</td>
<td>2012</td>
<td>Plos One</td>
<td>PV-IgG Anti-Dsg3 IgG Ak23</td>
<td>Kinases</td>
<td>Tyrosine kinase p38MAPK</td>
<td>Human keratinocyte cultures</td>
<td>Inhibition of tyrosine kinase using genistein and inhibition of p38MAPK using SB202190 prevented PV-IgG associated clustering of DSG3, loss of keratinocyte adhesion and fragmentation. Inhibition of tyrosine kinase and p38MAPK did not affect Ak23 activity.</td>
</tr>
<tr>
<td>Mao et. al&lt;sup&gt;46&lt;/sup&gt;</td>
<td>2014</td>
<td>J Invest Dermatol</td>
<td>PV-mAb</td>
<td>Kinases</td>
<td>MK2</td>
<td>Human keratinocyte cultures Mouse model</td>
<td>Inhibition and silencing of MK2 blocked PV mAb induced Dsg3 endocytosis and spontaneous blisters by PV-mAbs in mouse model.</td>
</tr>
<tr>
<td>Jolly et. al&lt;sup&gt;47&lt;/sup&gt;</td>
<td>2010</td>
<td>J Biol Chem</td>
<td>PV-IgG</td>
<td>Kinases</td>
<td>p38MAPK</td>
<td>Human keratinocyte cultures</td>
<td>Inhibition of p38MAPK using SB202190 in PV IgG treated cells resulted in DSG3 co-localized to the cell membrane and prevented PV-IgG induced redistribution of DsG-3.</td>
</tr>
<tr>
<td>Cirillo et. al&lt;sup&gt;48&lt;/sup&gt;</td>
<td>2014</td>
<td>Autoimmunity</td>
<td>PV-IgG</td>
<td>Kinases</td>
<td>Src</td>
<td>Human keratinocyte cultures</td>
<td>Inhibition of Src using Src-I-1, prevented phosphorylation of pkp3 and detachment from DSG3, resulting in a decrease in acantholysis in cells incubated with PV-IgG.</td>
</tr>
<tr>
<td>Author</td>
<td>Year</td>
<td>Journal</td>
<td>Condition</td>
<td>Kinase(s)</td>
<td>Inhibition Method</td>
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<tr>
<td>Cirillo et al.</td>
<td>2010</td>
<td>Exp Cell Res</td>
<td>PV Serum</td>
<td>PKC</td>
<td>Inhibition of PKC using Go6976 prevented complete depletion of Dsg 1, Dsg 3 and E-cadherin from surface of keratinocytes; preventing cadherin processing, recycling and depletion of cadherins from cell surface. PV induced loss of cell adhesion was also reduced.</td>
<td></td>
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<tr>
<td>Walter et al.</td>
<td>2017</td>
<td>Sci Rep</td>
<td>PV-IgG</td>
<td>PKC</td>
<td>Inhibition of kinases p38MAPK, Src, PKC using SB202190, PP2, Bim-X, blocked loss of cell cohesion induced by PV-IgG and Ak23. ERK inhibition U0126 was only protective against mucocutaneous PV-IgG.</td>
<td></td>
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<tr>
<td>Berkowitz et al.</td>
<td>2006</td>
<td>Proc Natl Acad Sci USA</td>
<td>PV-IgG</td>
<td>P38MAPK</td>
<td>Inhibition of p38MAPK using SB202190 in PV-IgG treated mice failed to develop blisters clinically and histologically.</td>
<td></td>
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<tr>
<td>Bektas et al.</td>
<td>2013</td>
<td>J Biol Chem</td>
<td>PV-IgG</td>
<td>EGFR</td>
<td>PV-IgG and AK23 induced ERK phosphorylation and blister formation was inhibited using EGFR inhibitor Ag1478. Such effects were also inhibited using upstream, p38MAPK inhibitor SB202190 which blocked PV-IgG induced phosphorylation of EGFR and as a result prevented keratinocyte acantholysis.</td>
<td></td>
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<tr>
<td>Spindler et al.</td>
<td>2010</td>
<td>J Immunol</td>
<td>PV-IgG</td>
<td>PKA, p38MAPK, cAMP</td>
<td>cAMP elevation via Iso blocked PV-IgG blister formation and promoted recovery of keratinocytes through PKA signaling in vivo. Incubation with Iso also blocked PV-IgG induced p38MAPK phosphorylation in both in vivo and in vitro.</td>
<td></td>
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<tr>
<td>Author et. al</td>
<td>Year</td>
<td>Journal</td>
<td>Autoantibodies</td>
<td>Kinases</td>
<td>Cell Type</td>
<td>Mouse Model</td>
<td>Conclusion</td>
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<tr>
<td>Spindler et. al</td>
<td>2013</td>
<td>J Clin Invest</td>
<td>PV-IgG Anti-Dsg3 IgG Ak23</td>
<td>Kinases</td>
<td>p38MAPK</td>
<td>Human keratinocyte cultures Mouse model</td>
<td>Tandem peptide prevented autoantibody induced p38MAPK activation and resulted in keratin filament retraction whilst promoting Dsg3 oligomerization.</td>
</tr>
<tr>
<td>Spindler et. al</td>
<td>2011</td>
<td>Am J Pathol</td>
<td>PV-IgG</td>
<td>Kinases</td>
<td>PKC</td>
<td>Human keratinocyte cultures Mouse model</td>
<td>Inhibition of PKC using Go6976, prevented suprabasal Dsg3 depletion in vitro and prevented blister formation in vivo.</td>
</tr>
<tr>
<td>Lanza et al</td>
<td>2008</td>
<td>J Biol Chem</td>
<td>PV Serum</td>
<td>Kinases</td>
<td>Cdk2</td>
<td>Human keratinocyte cultures Mouse model</td>
<td>Inhibition of Cdk2 using roscovitine prevented blister formation and acantholysis in mice injected with PV serum. In vitro, inhibition and knock-down of Cdk2 prevented PV induced cell detachment.</td>
</tr>
<tr>
<td>Spindler et. al</td>
<td>2014</td>
<td>J Invest Dermatol</td>
<td>PV-IgG Anti-Dsg3 IgG Ak23</td>
<td>Kinases</td>
<td>p38MAPK Plankoglobin Desmoplakin</td>
<td>Human keratinocyte cultures</td>
<td>Inhibition of p38MAPK using SB202190 in vitro incubated with AK23 reduced cell dissociation and prevented collapse of keratin filaments after plakoglobin silencing.</td>
</tr>
<tr>
<td>Vielmuth et al</td>
<td>2015</td>
<td>J Invest Dermatol</td>
<td>PV-IgG Anti-Dsg3 IgG Ak23</td>
<td>Kinases</td>
<td>p38MAPK</td>
<td>Human keratinocyte cultures</td>
<td>Inhibition of p38MAPK using SB202190, blocked AK23 and PV-IgG induced loss of cell cohesion and resulted in fragment numbers similar to that of the control.</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Year</td>
<td>Journal</td>
<td>Stimulation</td>
<td>Kinases</td>
<td>Kinase Inhibition</td>
<td>Effect</td>
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<tr>
<td>Waschke et al</td>
<td>2006</td>
<td>J Cell Biol</td>
<td>PV-IgG</td>
<td>Kinases</td>
<td>p38MAPK</td>
<td>Human keratinocyte cultures Inhibition of p38 MAPK using SB202190 blocked pemphigus IgG– triggered keratinocyte dissociation and prevented activation of Rho A.</td>
<td></td>
</tr>
<tr>
<td>Chernyavsky et al</td>
<td>2007</td>
<td>J Biol Chem</td>
<td>PV-IgG</td>
<td>Kinases</td>
<td>Src, EGFRK, p38MAPK</td>
<td>Human keratinocyte cultures Inhibition of Src using PP2 and p38MAPK using PD169316 blocked PV-IgG-dependent cell shrinkage and keratin aggregation in vitro. PP2 also decreased EGFRK and p38MAPK signaling.</td>
<td></td>
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<tr>
<td>Rotzer et al</td>
<td>2014</td>
<td>J Biol Chem</td>
<td>PV Serum</td>
<td>Kinases</td>
<td>p38MAPK</td>
<td>Human keratinocyte cultures Inhibition of p38 MAPK using SB202190 or activation of RhoA blocked PV-IgG-mediated loss of cell adhesion.</td>
<td></td>
</tr>
<tr>
<td>Cirillo et al</td>
<td>2008</td>
<td>Int J Immunopathol Pharmacol</td>
<td>PV Serum</td>
<td>Kinases</td>
<td>Kinases</td>
<td>Human keratinocyte cultures Broad kinase inhibitor, STS decreased PV-specific phosphorylation and dramatically reduced acantholysis in vitro in presence of PV serum.</td>
<td></td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>Journal</td>
<td>Model</td>
<td>Endogenous Products</td>
<td>Results</td>
<td></td>
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<tr>
<td>Wei et al. 64</td>
<td>2021</td>
<td>BioMed Research International</td>
<td>PV Serum</td>
<td>Kinases Trx2, ASK1</td>
<td>Human keratinocyte cultures Mouse model  Inhibition of ASK1 using GS-444217 in PV treated cells resulted in reduction of apoptosis while silencing of Trx2 increased apoptotic rate. In mice treated with Trx2 over expressing vector, acantholysis was also reduced.</td>
<td></td>
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<tr>
<td>Murrell et al. 84</td>
<td>2021</td>
<td>Br J Dermatol</td>
<td>PV-IgG</td>
<td>Kinases BTK</td>
<td>In vivo human patient  Inhibition of BTK using rilzabrutinib (PRN1088) in patients with PV, resulted in control of disease activity in 4 weeks.</td>
<td></td>
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<tr>
<td>Esaki et al. 66</td>
<td>1995</td>
<td>J Invest Dermatol</td>
<td>PV-IgG</td>
<td>Hydrolytic Enzyme Phospholipase C DJM-1 Squamous carcinoma cell line</td>
<td>Incubation of DJM1 cells with 10% PV serum and U73122 PLC inhibitor (10um) results in inhibition of PV-IgG-Induced detachment of cell-cell contacts</td>
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<tr>
<td>Sanchez Carpintero et. al 67</td>
<td>2004</td>
<td>British Journal of Dermatology</td>
<td>PV-IgG</td>
<td>Hydrolytic Enzyme Phospholipase C Calmodulin Mouse model</td>
<td>Inhibitors were injected 3hr before intradermal injection of PV-IgG PLC inhibitor U-73122, (100 lg g) Pre-treatment of mice with inhibitors of intracellular signalling cascades prevented PV IgG-induced blister formation in vivo Treatment with calmodulin inhibitor (W7-Hydrochlorine) prevented acantholysis</td>
<td></td>
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</tr>
<tr>
<td>Marquina et al. 42</td>
<td>2008</td>
<td>Br J Dermatol</td>
<td>PV-IgG</td>
<td>Other Molecules NOS Tyrosine-Kinase NF-kb Mouse model</td>
<td>Pre-treatment with inhibitors of TK, nNOS and nonselective NOS, completely prevented NTR expression and clinical and histological presentation of PV in mice.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>Journal</td>
<td>PV-IgG</td>
<td>Other Molecules</td>
<td>Main Molecules</td>
<td>Cell Type</td>
<td>Summary</td>
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<tr>
<td>Espana et al</td>
<td>2013</td>
<td>Experimental Dermatology</td>
<td>PV-IgG</td>
<td>nNOS</td>
<td>Mouse model</td>
<td>PV IgG increased nNOS in basal cells of epidermis and highlighted increased expression in cytoplasm during blistering Pre-treatment with nNOS inhibitors decreased acantholysis</td>
<td></td>
</tr>
<tr>
<td>KalantariDehagi et al</td>
<td>2013</td>
<td>The Journal of Biological Chemistry</td>
<td>PV Serum</td>
<td>Mitochondria &amp; ROS</td>
<td>Cell line (Het-1A) Mouse model</td>
<td>Mitochondrial protecting drugs (minocycline, nicotinamide, cyclosporine) abolished acantholysis</td>
<td></td>
</tr>
<tr>
<td>Liang et al</td>
<td>2017</td>
<td>Biomedicine and Pharmacotherapy</td>
<td>PV Serum</td>
<td>NOD2 &amp; NF-kB</td>
<td>Human keratinocyte cultures</td>
<td>Naringenin reduces cell-cell detachment induced by PV serum</td>
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<tr>
<td>Lotti et al</td>
<td>2018</td>
<td>Frontiers in Immunology</td>
<td>PV-IgG</td>
<td>FasL</td>
<td>Mouse model</td>
<td>Administration of Anti-FasL Ab after PV IgG injection prevents acantholysis in cultured keratinocytes Magnitude of acantholytic areas significantly decreased and PV IgG injection failed to induce activation of caspase-8 in FasLΔs/Δs mice, but not in FasLΔm/Δm mice.</td>
<td></td>
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<tr>
<td>Chernyavsky et al</td>
<td>2008</td>
<td>J Biol Chem</td>
<td>PV-IgG</td>
<td>Alpha 7</td>
<td>Human keratinocyte cultures</td>
<td>AR-R17779, AChR inhibitor decreased PV-IgG induced activity and reduced acantholysis.</td>
<td></td>
</tr>
</tbody>
</table>
PV-IgG

Other Molecules, Hydrolytic enzymes

IP3R
CRAC
Phospholipase C

Human keratinocyte cultures
Human skin ex vivo

PLC, IP3R and CRAC inhibitors highlighted decreased Ca2+ influx in PV-IgG treated in-vitro cells. Blistering was ameliorated in ex-vivo samples when treated with these inhibitors.

Table 2. Distribution of studies that used in vitro, ex vivo (skin cultures) and in vivo (mouse models).

<table>
<thead>
<tr>
<th>Subject</th>
<th>In vitro</th>
<th>Ex vivo</th>
<th>In vivo</th>
<th>In vitro and ex vivo</th>
<th>In vitro and in vivo</th>
<th>In vivo, in vitro and ex vivo</th>
<th>PV Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV Serum</td>
<td>Caspases (1)</td>
<td>Proteases (3)</td>
<td>Proteases (1)</td>
<td>Kinas (2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proteases (1)</td>
<td>Kinases (3)</td>
<td>Other molecules (2)</td>
<td>PV-IgG</td>
<td>Kinases (1)</td>
<td>Proteases (4)</td>
<td>Other molecules (2)</td>
</tr>
</tbody>
</table>
Legends

Figure 1: Flow chart of the selection process according to PRISMA guidelines.

Table 1. Pathogenic signalling mechanisms in PV – caspases and apoptosis, proteinases, kinases, hydrolytic enzymes and other molecules.

Table 2. Distribution of studies that used in vitro, ex vivo (skin cultures) and in vivo (mouse models).
Records identified through database searching: PubMed and medRxiv (n=882)

Records excluded (n=238) Reasons: Review articles and language other than English (LOTE)

Records screened for eligibility criteria (n=644)

Records excluded (n=485) Reasons: Article (title and abstract) not relevant to PV

Full text articles assessed for inclusion criteria (n=159)

Records excluded (n=103) Reasons: No evidence of pathogenicity, only immunohistochemistry data, efficacy of proposed treatments not shown

Studies included in the qualitative synthesis (n=56)
Author/s:
Kaur, B; Kerbrat, J; Kho, J; Kaler, M; Kanatsios, S; Cirillo, N

Title:
Mechanism-based therapeutic targets of pemphigus vulgaris: A scoping review of pathogenic molecular pathways

Date:
2021-09-02

Citation:

Persistent Link:
http://hdl.handle.net/11343/298933