

Review Article

doi:10.34172/ajpr.2021.06



Exploring RNAs Interactions and Polymorphisms in the Pathophysiology of Pemphigus: A Review

Ali Bahrami^{1,2}, Mohammad Taheri³, Parisa Habibi⁴, Meysam Soleimani^{2*}, Fatemeh Nouri^{2*}

¹Student Research Committee, Hamadan University of Medical Sciences, Hamadan, Iran

²Department of Pharmaceutical Biotechnology, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran

³Department of Medical Microbiology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

⁴Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran

***Corresponding author:**

Fatemeh Nouri,
Meysam Soleimani
Ph.D. in Pharmaceutical
Biotechnology
Hamadan University of
Medical Sciences
Hamadan, Iran.
Tel: +98-9188596549,
Email: Fatemenouri1@gmail.
com,
meysambadia@yahoo.com

Abstract

Background: Pemphigus consists of a group of rare autoimmune blistering diseases involving the skin and mucous membranes. Pemphigus pathophysiology is mediated by autoantibodies against two desmosomal cadherins, namely, desmoglein (Dsg) 1 and (Dsg) 3 that are present in the skin and mucosal membranes. The involvement of coding and non-coding RNAs in the pathophysiology of pemphigus has been studied in the literature. MicroRNAs are small RNAs that could also be used as diagnostic biomarkers for some autoimmune diseases. The aim of this research was to explore the potential of this specification of some RNAs to be used as biomarkers for diagnosing pemphigus or its severity. This review discussed RNA expressions in patients with pemphigus.

Methods: A comprehensive search was performed on published studies from 1990 to May 2020 using different search engines including PubMed, Scopus, and Web of Science.

Results: In general, 335 articles were obtained according to search keywords. Then, 41 relevant studies were selected based on the inclusion and exclusion criteria. MiR-338-3p, miR-424-5p, and miR-584-5p were among the miRNAs that were reported to be increased in pemphigus. The C3 mRNA, mRNA of CD36, mRNA of CD163, mRNA of urokinase plasminogen activator (PA), IL23R mRNA, RORγt mRNA, and human leukocyte antigen G1 (HLA-G1) mRNA were coding RNAs that increased in pemphigus in addition to the activity of the mRNA of tissue-type PA while HLA-G2 mRNA decreased in pemphigus.

Conclusion: Overall, this study investigated the role of Mir-338-3p, miR-424-5p, MiR-127, miR-584-5p, and some mRNAs in pemphigus, and it was revealed that some RNAs may be impressive on pemphigus. More studies and clinical assessments need more information about the role of RNAs on pemphigus to obtain a better view of their mechanisms and use them as biomarkers for earlier diagnosis or probable treatment.

Keywords: Pemphigus, RNA, miRNA, Cytokine, Gene expression

Received 3 April 2021, Accepted: 30 May 2021, ePublished: 20 August 2021



Introduction

Pemphigus is an autoimmune blistering disease in which autoantibodies against two desmosomal adhesion proteins (i.e., Dsg1 and Dsg3) cause pathogenicity (1,2). Desmoglein (Dsg) 1 and 3 are the main components of desmosomes, which are involved in connecting the keratinocytes (3). The autoantibodies in this disease promote the hydrolysis of plasminogen into plasmin in keratinocytes and cause the loss of cell-cell adhesion, leading to the appearance of blisters (4). Different clinical appearances of the disease are due to dissimilar specific antigens and the distribution of these antigens in various regions of the body and separate layers of the epidermis (5). Four types of pemphigus are known, including pemphigus vulgaris (PV), pemphigus vegetans, pemphigus foliaceus, and paraneoplastic pemphigus, and PV is the most common and severe one (6).

The central dogma of molecular biology is that DNA makes RNA and later makes proteins (7). RNAs are genetic sequence transcripts from DNA and are information storages and act as templates that catalyze the synthesis of complex molecules (8). RNAs have different functions such as carrying information to ribosomes, transferring amino acids to ribosomes, interfering in the expression of genes, and the like. MicroRNAs have ~22-nucleotide and are single-strand RNAs. They further inhibit the expression of specific mRNA targets through Watson-Crick base pairing between the miRNA 'seed region' and sequences commonly located in the 3' untranslated regions (UTRs) of mRNA. It has been estimated that up to 1000 miRNAs are encoded by the human genome (9). The evolution of miRNA begins in the nucleus with transcription by RNA polymerase II, and the aborning transcript is pri-miRNA, which is a double strand that can be as long as several

kilobases. The processing of miRNA begins in the nucleus with cleavage by Drosha (RNase III-like enzyme) and its co-factors. Then, the miRNA is nearly 70 nucleotides and is exported from the nucleus to cytoplasm by the export factor Exportin 5. In the cytoplasm, Dicer (another RNase III-like endonuclease) creates ~22 nt RNA duplex, and then the miRNA will be a single strand by the final complex RNA-induced silence complex (10). Recent research indicated that small RNA molecules from short-interfering RNAs to microRNAs are also capable of moving between cells and through the vasculature (11). RNAs have a role in the pathogenesis of pemphigus. Accordingly, this review focused on understanding the role of each RNA and how the changing of RNA expression, especially miRNAs, influences the pathogenesis of PV aiming at using it for treating and controlling the disease (Figure 1).

Methods

This review aimed to determine the interaction between Pemphigus disease and RNA molecules. Some electronic databases, namely, the external databases of PubMed, Scopus, Web of Science, and open access Journal Directory (from 1990 until May 2020) were searched to find related articles. Our search strategy was accomplishing primarily mini-review use of English sensitive keywords with any probable mesh of keywords such as 'pemphigus AND miRNA', 'pemphigus AND micro AND RNA', 'pemphigus AND lncRNA', 'pemphigus AND lnc AND RNA', 'pemphigus AND piRNA', and 'pemphigus AND sno and RNA'. Other keywords were 'pemphigus

AND snoRNA', 'pemphigus AND snRNA', 'pemphigus AND sn AND RNA', 'pemphigus AND miRNA AND review', 'pemphigus AND microRNA AND review', and 'pemphigus AND RNA' which were searched in the titles and abstracts of studies. Furthermore, the reference list of the articles was evaluated to increment the sensitivity and choice of most literature which could not be identified in the database. Initiating with the greatest sensitivity search, 335 articles on external databases were found and collected by a researcher using Endnote software. Then, the articles from all the cited databases were unified and duplicate articles were removed from further analysis. Two researchers separately investigated all the articles and excluded the ones that were unrelated to the topic and the inclusion index criteria (search keywords). Several articles and abstracts were excluded after reviewing the titles. The extant articles were cautiously evaluated, and relevant studies were selected accordingly. Eventually, 41 articles were analyzed after obtaining related articles and applying the limitations of the search strategy. During the review of the articles in 2020, new articles were included in our study, if any (Figure 2).

Results

RNA Interference in Pemphigus

The primary signs in most of the pemphigus cases are oral lesions and then cutaneous lesions (2). The pemphigus auto-antibodies induced losing cell to cell connection. The inhibition of EGFR by shRNA causes the prevention of the internalization of Dsg and retraction of keratin

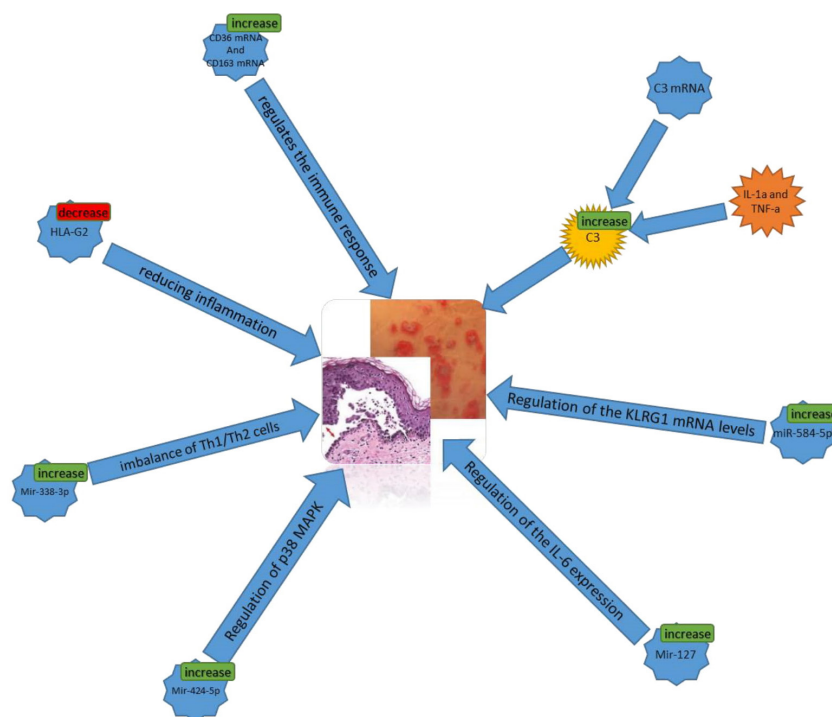


Figure 1. Graphical Abstract of the Present Study.

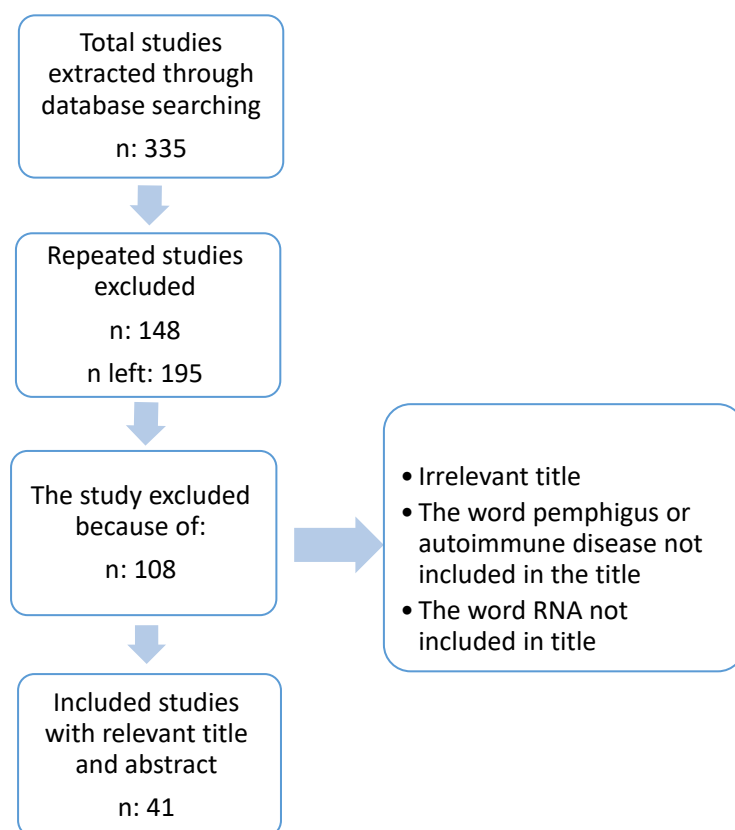


Figure 2. Flowchart of Search Results in Search Engines.

intermediate filaments. The PV immunoglobulin G (IgG) will be prevented from blistering in a mouse model using EGFR inhibitors and shRNA that inhibit EGFR (12). Thus, the therapeutic intervention of iRNAs could have good outcomes in the prognosis of pemphigus the treatment of which is difficult (13).

miR-217

Overall, 65 patients with oral PV and 38 healthy people were selected in this study, and their oral mucosal tissues, serum, and saliva were collected based on the aim of the study. Interleukin-6 (IL-6) is an important factor in immune responses that is the target of miR-217 and has an important function in immune responses, inflammation, cell differentiation, coagulation, and the development of a tumor. The tissue macrophages secrete IL-6 to promote the maturation of pre-B cells. In addition, IL-6 increases inflammatory responses induced by damage, trauma, stress, and infection. It increased in the oral mucosal tissues of a patient with PV compared with the controls. Moreover, changes in the IL-6 gene expression were found in the serum and the saliva. This upregulation of IL-6 can be used as a biomarker for PV, especially at the early stage. In this study, a binding site of miR-217 at the 3'UTR of IL-6 mRNA was reported thus miR-217 is a regulator of IL-6 expression and might affect the PV pathology by IL-6 expression regulation (6).

Many rare diseases are diagnosed lately, probably leading to severe, irreversible, debilitating, and life-threatening consequences. Approximately 30% of rare diseases have been guesstimated that have no specific diagnosis. Accordingly, exact and timely diagnosis is often important for prevention and accurate treatment. Genetic factors such as several genes or chromosomal abnormalities are the cause of nearly 80% of rare diseases and these agents can be used as biomarkers since the regulation of many diseases is associated with genetic and epigenetic factors (14). Biomarkers are biological parameters that can be measured and cause choosing the appropriate and often personalized medicine. Biomarkers should be easily accessible thus it is acceptable if they can be polled from body fluids such as serum or urine (15). In general, 1500 transcribed miRs were identified since the discovery of miRs (14). miRs are now used as biomarkers for diseases such as cancer and some specific miRs are involved in the pathogenesis of these diseases (16,17). miRs can further be applied as almost accessible biomarkers for diagnosing rare diseases (14).

miR-338-3p

It was demonstrated that the levels of Th2 cells and Th2-type cytokines (IL-4 and IL-10) increase while the levels of Th1 cells and Th1 cytokines (Interferon-gamma [IFN- γ] and IL-2) decrease in PV patients, thus the TH1/Th2

balance in the peripheral blood plays the main role in the immunopathogenesis of PV patients (18). miR-338-3p may contribute to the synthesis of the Dsg3 antibody. The expression of the miR-338-3p increases in PV patients compared with controls and its expression has a positive correlation with the pemphigus area and activity score and anti-Dsg3 antibody titers (18). In a study by Lin et al on 42 patients and 33 healthy people, it was reported that the increased expression of miRNA-338-3p changed and decreased after effective treatment. It was shown that the severity of pemphigus is related to the level of miR-338-3p. No difference was observed between people with pemphigus for the first time and people who had relapsed disease (19).

The decline in IFN- γ and IL-4 levels while a rise in the IL-10 level, along with the increased expression of miR-338-3p implies that the imbalance of Th1/Th2 cells is related to the altered expression of miR-338-3p. MiR-338-3p causes an imbalance of Th1/Th2 cells thus it plays a role in the production of the Dsg3 antibody in PV patients. TNFRI-associated death domain protein (TRADD) is a target gene of miR-338-3p and an adaptor molecule that induces either apoptosis or proliferation. The protein expression of TRADD decreases in PV patients and is because of the overexpression of miR-338-3p, meaning that miR-338-3p causes the imbalance of Th1/Th2 cells directly by suppressing the function of TRADD (18). RNF114 is another target gene of miR-338-3p and promotes T-cell activation thus it can take part in the regulation of immunity and has a role in pemphigus pathogenesis. RNF114 down-regulates in both mRNA and protein levels when miR-338-3p increases in vitro while in vivo, its mRNA and protein are higher in patients compared to healthy subjects. It was suggested that RNF114 increases through other pathways in pemphigus patients (19).

miR-424-5p

Autoantibodies against Dsg 1 and 3 promote the hydrolysis of plasminogen into plasmin in keratinocytes. They further cause the loss of cell-cell adhesion and make blisters. Humoral responses, regulated by CD4⁺ T cells, may contribute to the pathogenesis of pemphigus. Pro-inflammatory T cells increase the humoral responses whereas regulatory T cells (Tregs) inhibit B-cell activation and antibody production. Some miRNAs modulating T- and B-cell function can regulate the development of autoimmune diseases (AIDs). Three patients with pemphigus and three healthy subjects were selected in miRNAs microarray analysis. To assess the similarity, the total RNA was extracted from peripheral blood mononuclear cells (PBMCs), and miRNA expression profiles between the patients and healthy subjects were analyzed by the principal-component analysis. There was a clear difference between the two groups and similarity in each group. There were 71 ($P < 0.05$, fold change

> 2) upregulated and 53 ($P < 0.05$, fold change < 0.5) downregulated miRNAs, respectively.

miR-424-5p was one of the miRNAs which was upregulated up to more than 1,000-fold in the patients. The expression of miR-424-5p in the PBMCs of 9 other patients with the same age and gender as healthy subjects was defined by the real-time quantitative polymerase chain reaction. The levels of miR-424-5p in the PBMCs in the patients were higher compared to healthy subjects. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes analysis showed that the potential targeted genes by miR-424-5p may regulate a wide range of pathways including the p38 and another mitogen-activated protein kinase (MAPK). In pemphigus, although the signaling pathway of MAPK is dysregulated, miR-424-5p targets this signaling pathway and may adjust the pathogenesis of pemphigus (4). Specific autoantibodies of pemphigus can induce the p38 MAPK, which is associated with the collapse of the cytoskeleton, separation of desmosomes, and apoptosis of keratinocytes. The inhibition of p38 MAPK in keratinocytes can prevent pemphigus-specific autoantibodies, inducing the reorganization of cytoskeletons and phosphorylation of heat shock proteins (HSP) 27. HSP27 phosphorylation has been found in pemphigus patients' biopsies and may involve in cytoskeletal regulation. Cytoskeletal dysregulation is associated with the loss of cell-cell adhesion. Skin blistering was prevented by inhibiting pemphigus IgG-activated signaling in epidermal cells in a mouse model of pemphigus. It is believed that miR-424-5p is involved in blister formation and regulation of p38 MAPK activation and HSP27 phosphorylation during the development of pemphigus.

Long Non-coding RNA Polymorphisms (single nucleotide polymorphisms, SNPs) in Pemphigus

Generally, 229 patients with endemic pemphigus foliaceus (EPF) and 6681 healthy controls were used, and the genotype and allele frequencies of 2080 SNPs in their whole genome were collected based on the study aim. It should be noted that rs7195536, rs6095016, rs6942557, rs1542604, and rs17774133 are exonic SNPs considering that they may change the secondary structure of lncRNAs and their changes may influence some gene expressions and regulations (20). It is known that the function of RNAs depends on their structures. Four SNPs (i.e., rs7144332, rs1542604, rs6942557, and rs17774133) are associated with lncRNAs and involved in EPF. These SNPs exist in introns or spliced exons rather than mature lncRNAs. SNPs on lncRNA genes can cause interference not only in the post-transcriptional adjustment of other gene expressions but also in their transcriptional regulation. lncRNA AC009121.1, which is associated with SNP (rs7195536), is an expression quantitative trait locus (eQTL) for three relative genes including RMI2, RP11485G7.5

(AC009121-2), and CTD- 3088G3-4 (AC099489-2), implying that lncRNA AC0091211 may regulate these three genes, and SNP rs7195536 can make some changes in its regulation. It has been suggested that this SNP may have a direct effect on EPF capacity (21).

The IL23/Th17 pathway is vital in tissue immune-surveillance and autoimmunity mechanisms and is also involved in the pathogenesis of some AIDs. Four SNPs in the IL23R gene were analyzed, among which only rs11209026>G was associated with PF susceptibility. This SNP causes replacing the Arg with Gln residues and this change may result in IL23R function. In this study, 115 patients with PF and 201 healthy people were selected for analysis. A slight increase in IL23R mRNA expression was observed in PF patients compared to the controls. Additionally, the level of mRNA was higher in patients with chronic PF compared to new patients. In addition, the increased expression of proinflammatory cytokine IL23 was reported in PV (22) instead of PF.

The leukocyte-associated Ig-like receptor 1 (LAIR-1) is expressed in most PBMC, is highly important for adjusting the immune response, and is a collagen-binding inhibitory receptor. LAIR-2 is connected with the same ligands and antagonizes the function of LAIR-1. Both LAIR-1 and LAIR-2 are Ig receptors. The LAIR1 and LAIR2 SNPs were studied if they are associated with PF susceptibility. C1q (the first member of the complement) exists in the intercellular space of PV lesions (23) and binds with LAIR-1, and this connection inhibits some immune responses such as the activation and differentiation of monocytes to dendritic cells (DC) and the production of IFN- α by plasmacytoid DCs. LAIR-2 can also return the differentiation of monocytes to DC and the production of IFN- α (24). Likewise, collagen XVII binds LAIR-1 and LAIR-2. The autoantibodies of blistering AIDs, including PV and PF target collagen XVII (25). The connection of autoantibodies with collagen XVII can affect the binding of collagen and LAIR-1 thus affecting the immune response in these diseases (26). LAIR1 SNP rs56802430 is associated with LAIR1 expression and PF susceptibility but its association with PF is due to other possible functions instead of differential expression of LAIR1 mRNA. Moreover, rs11084332 is associated with PF. Some of the SNPs related to LAIR-1 mRNA expression or PF susceptibility are present in 3'UTR, which is a binding site for miRNAs. SNPs rs74463408 and rs3745444 related to LAIR-1 mRNA expression and SNP rs11084332 are associated with PF that is present in that region. The 5'UTR SNP rs2287828 of LAIR2 is only associated with PF among the eight investigated LAIR2 SNPs. Some SNP-SNP interactions in LAIR2 include rs2287828 interaction with rs2042287 and rs114834145, and that of rs114834145 with rs2277974. SNP rs2287828 does not influence gene expression, thus indicating that differential mRNA levels of LAIR2 should not involve in PF. Nonetheless, these four

interactions organize a haplotype that is associated with PF susceptibility and causes the upregulation of LAIR2 in healthy controls, and finally, higher mRNA levels of LAIR2 might affect susceptibility to PF (27).

Nine 3'UTR SNPs were evaluated, which were distributed among six immune-related genes. A different expression of these genes was found in PF and there was an association between PF and the A/G genotype of KLRG1 rs1805672. A single miRNA-mRNA interaction was studied, and the overlapping of rs1805672 of KLRG1 with the miRNA targeting site was observed accordingly. The heterozygous genotype of the KLRG1 rs1805672 is associated with an increased predisposition to PF, and there is a positive association between KLRG1 mRNA and miR-584-5p levels. MiR-584-5p is an intronic miRNA and located in chromosome 5 (not in the same chromosome of KLRG1) as an intron of the SH3 domain and tetratricopeptide repeats 2 genes (SH3TC2). In this study, a positive correlation was observed between the mRNA of SH3TC2 and miR-584-5p and KLRG1 in the same PBMC samples. The nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappa B) signaling upregulated the expression of miR-584-5p. Furthermore, NF-kappa B upregulates the KLRG1, which is the target of miR-584-5p. The rs1805672 G allele can cut the binding of this microRNA. It is known that KLRG1 and SH3TC2 transcriptions are concordantly regulated by NF-Kappa B. It is possible that the KLRG1 protein synthesis harboring the rs1805672 G allele after the activation of NF-kappa B is not affected by miR-584-5p. The rs1805672 G allele cancels the miR-584-5p mediation thus this SNP may play an important role in regulating KLRG1 mRNA levels. Considering that KLRG1 mRNA levels increased in PF patients (compared with controls), it should be noted that rs1805672 essentially contributes to the pathogenesis of PF. Several other SNPs in the 3'UTR of KLRG1 may involve in the regulation of KLRG1 post-transcriptionally caused by miRNA, thus they may associate with an AID such as pemphigus. High levels of KLRG1 proteins impair E-cadherin-dependent cell-cell adhesion. Therefore, KLRG1 proteins have a role in the pathogenesis of pemphigus (28).

Genetic Variants Associated With AIDs Affecting Putative microRNA Binding Sites

Based on the evidence, more than 90% of genetic variations are related to AIDs, which are in the non-coding regions of the human genome and specifically in the regulatory sequences. SNPs in the 3'UTR of mRNA (miRSNP) can change the miRNA binding site and affect regulation by miRNA, thus causing AIDs. In this study, 34 miRSNPs were identified, which may affect the binding site of 86 miRNAs in 18 target genes. The PTPN2 gene is associated with five diseases and has two miRSNPs in its 3'UTR. The risk allele (T) of rs60474474 disrupts the binding of miR-4290, and the protective allele (G) of rs45450798 located in

the 3'UTR of PTPN2 creates a binding site for miR-4531. In mice, the lack of PTPN2 causes the perturbations of T cell tolerance and increased T cell and B cell responses, resulting in severe inflammatory and autoimmunity disease. The repression of PTPN2 by 3'UTR SNPs is related to several diseases. The risk allele eventually decreases PTPN2 expression and causes AIDs. MiR-4290 is not present in any blood cell type or whole blood thus the increased expression of miR-4290 due to reduced PTPN2 can be used as a biomarker to immune-related diseases. It has been shown that SNPs associated with complex diseases are more likely to be an eQTL compared to non-related SNPs. Approximately 12% of causal non-coding SNPs in AIDs are eQTL, and 28 miRSNPs associated with AID that is eQTL for their target genes, were discovered as well (29).

Loss of Flotillin Expression in PV

Flotillin-1 and -2 are proteins that are present in various cellular compartments and are associated with lipid microdomains called rafts. Various cell adhesion proteins such as E-cadherin, β -catenin, and p120 catenin are related to flotillins. It is noteworthy that flotillins directly interact with plakoglobin, which is localized in both adhesion junctions (AJs) and desmosomes thus it is reasonable that flotillins may be involved in desmosomal adhesion in the epidermis tissue. The absence of flotillin decreases the expression of Dsg3 in the HaCaT human keratinocyte cell line and it is due to the increased Dsg3 lysosomal turnover. Flotillin ablation harms desmosomal adhesion strength. Accordingly, microRNAs interfering in the expression of flotillins have also an important role in the pathogenesis of PV and can be used as a candidate for the treatment of PV (30).

Complement Activation in Pemphigus

The complement system is one of the main factors that is involved in acantholysis in pemphigus. Total hemolytic complement reduces in the blister fluids of pemphigus thus it shows a local activation of complement in pemphigus. The activation of complement causes a group membrane attack complex that is merely found in PV lesions rather than the unaffected skin, highlighting the role of complement in acantholysis. Research findings indicated that PV lesions still develop in the lack of C3 or C5, showing that the role of complement is not critical in PV but it increases the development of blisters. PV-IgG antibodies cause the expression of C3 mRNA in keratinocytes, and acantholysis reduces about 43% by using anti-C3 antibodies. Antibodies against C3 do not affect C3 mRNA, thus the regulation of C3 is mediated via other pathways instead of in an autocrine manner. Pro-inflammatory cytokines (i.e., IL-1 α , TNF- α , and IL-6) increase complement synthesis in different cell lines. IL-1 α and TNF- α induce C3 synthesis in keratinocytes, leading

to the reduction of acantholysis by blocking the activity of both cytokines and downregulation of C3 mRNA. The lack of the complete inhibition of C3 mRNA means that other mediators play a role in C3 activation. More precisely, C3 is upregulated by IL-1 α and TNF- α and is involved in the acantholysis process in PV (31).

Contribution of T helper Type 1 to the Pathogenesis of PV

T cells play an essential role in the progression of PV similar to any other AIDs. The presence of both TH1-related and TH2-related immune responses simultaneously causes the high titer of autoantibodies in this disease. The $\gamma\delta$ T cell is the component of innate immune cells and it is about 1-10% of the total T-cells in circulation. $\gamma\delta$ T cell expresses the $\gamma\delta$ T cell receptor (TCR). These cells are involved in immune surveillance at the mucosal and epithelial surface. Several AIDs and inflammatory conditions are related to the abnormal function of $\gamma\delta$ T cells. The findings of this study revealed that the level of IFN- γ is higher in the serum of PV patients, and the serum level of IL-4 increases, which might be because of the secretion of IL-4 by other immune cells in the circulation. The increased frequency of circulating $\gamma\delta$ T cells is observed in patients with PV compared with the controls. The anti-Dsg3 level represents a higher correlation ($r=0.951$, $P<0.0001$) with $\gamma\delta$ T cells in comparison with the anti-Dsg1 level ($r=0.337$, $P<0.06$). IFN- γ producing $\gamma\delta$ T cells could increase the frequency in PV patients compared with controls while the IL-4 producing $\gamma\delta$ T cell fraction decreased in patients. Pattern-recognition receptors known as scavenger receptors are involved in the immune response of patients suffering from PV. CD36 is a class B and CD163 is a class I receptor. These receptors are also expressed on the $\gamma\delta$ T cells surface, respond to the TCR signal strength, and regulate the immune response. The CD36 mRNA expression increased in isolated blood $\gamma\delta$ T cells and the patients' tissues whereas CD163 was only increased in the tissues (32).

Tissue-Type Plasminogen Activator and Cytokines

Tissue-Type Plasminogen

Plasminogen activator (PA) catalyzes the conversion of the plasminogen to plasmin (a kind of proteinase) and is involved in some of the cutaneous disorders. Tissue-type PA (tPA) activity increases in the lesional epidermis of patients with psoriasis, pemphigus, bullous pemphigoid, and Hailey-Hailey disease compared with normal people. On the other hand, tPA has not been immunocytochemically detected in the normal epidermis. Moreover, tPA mRNA is presented in the lesional epidermis of patients while not existing in the normal epidermis. Therefore, the presence of tPA mRNA may be associated with the regenerative process in the tissue (33).

Cytokines

Complement and protease secretion have a role in the pathogenesis of acantholysis in PV. In addition, cytokines such as IL-1 α and TNF- α are involved in the regulation and synthesis of complement and proteases. These cytokines induce keratinocytes C3 synthesis and pro-inflammatory cytokines. IL-1 β and TNF- α induce the synthesis of urokinase PA (uPA) and urokinase PA receptor (uPA-R). The main source of cytokines in the epidermis are keratinocytes, indicating that cytokines play a role in bullous pemphigoid (34), and using antibodies against IL-1 or IL-6 of mice completely blocks the development of blisters. Additionally, the levels of IL-6 and TNF- α have been increasingly reported in the sera of PV patients (35), and the expression of these cytokines could not be specific to PV because it was detected in many inflammatory and bullous disorders (36). In a study by Anhalt et al, PA activity was inhibited by dexamethasone rather than acantholysis (37). According to Theda Schuh, PA system inhibitors could not prevent acantholysis mediated by PV-IgG (38). Thus, these studies show that the plasminogen/plasmin system is not crucial for the development of pemphigus (39). IL-1 α and TNF- α derived from keratinocytes increase acantholysis both *in vivo* and *in vitro*. These cytokines increase the mRNA expression of uPA and C3, which are involved in PV pathogenesis, and their *in vitro* inhibition suppresses acantholysis. However, they are not necessary for the pemphigus development *in vivo*.

A slight amount of ROR γ t, which is a specific transcription factor of Th17 cells, was found in the skin biopsies of PF patients similar to healthy controls. Conversely, the level of ROR γ t mRNA in the PBMCs of PF patients was higher compared to the controls. This irrelevance is probably because of the samples used for the measurement of mRNA or the small group size of samples. The level of IL17⁺ cells in PV patients' skin biopsies was higher and about 5.2% (40). There was a relationship between the expression of ROR γ t and IL23R, and the induction of ROR γ t expression depends on the expression of IL23R. The differentiation of Th17 cells related to some cytokines (e.g., TGF- β , IL6, IL1, IL21, and IL23) is necessary for the maintenance and the proliferation of Th17 cells (41).

Human leukocyte Antigen-G Expression in PV

HLA-G is a non-classical HLA class I molecule that has a role in the induction of tolerance. It is different from its classical peers because its gene has a few polymorphisms and is expressed in low values (42). PV is associated with tolerance induction mechanisms. It is evident that the transcription of HLA-G1 increases while that of HLA-G2 decreases in PV patients compared to healthy controls. HLA-G mainly contributes to the safekeeping of cells or tissues from the demolition of the natural killer or inflammatory cells. HLA-G may also have inhibitory functions such as prostaglandin E2 or transforming growth

factor- β that are both produced in the keratinocytes of the epidermis and involved in the immunohomeostatic balance of the skin at the end of inflammation (43). It is suggested that HLA-G has a key role in reducing inflammation and its level is different between PV and healthy people (44). The manuscript summary is presented in Table 1.

Discussion

The autoimmune blistering disease pemphigus affects a group of people worldwide and decreases their quality of life. The pathology of pemphigus is associated with autoantibodies against adhesion protein Dsg 1 and 3 existing in the epidermis. Although the actual cause of pemphigus is still unknown, it has been observed that some RNA interactions are involved in the pathogenicity of pemphigus. MiR-338-3p expression increases in pemphigus patients and has a positive effect on anti-Dsg3 antibody titers. More expressions of MiR-338-3p cause an increase in the level of IL-4 and IL-10 whereas a decrease in the level of IFN- γ , and these changes cause an imbalance of Th1/Th2 cells involving in the pathogenicity of pemphigus. MiR-424-5p levels increase in pemphigus patients and affect p38 MAPK activation and HSP27 phosphorylation thus causing blistering. It was found that IL-6 increases in the oral mucosal tissues of PV patients. MiR-217 binds to the 3'UTR of IL-6 mRNA thus it can affect the pathogenicity of pemphigus. Rs1805672 G allele inhibits the miR-584-5p mediation, and KLRG1 is a target of miR-584-5p that increases in PF patients. Accordingly, Rs1805672 G can be involved in the pathogenicity of pemphigus. C3 mRNA synthesis induces by IL-1 α and TNF- α , increases in pemphigus patients, and causes acantholysis. The expression of CD36 mRNA increases in $\gamma\delta$ T cells and the patients' tissues whereas CD163 mRNA only increases in the tissue. The level of anti-Dsg3 is associated with $\gamma\delta$ T cells thus CD36 is involved in the pathogenicity of pemphigus. Totally, C3 mRNA, mRNA of CD36, and mRNA of CD163 are coding RNAs that increase in pemphigus. Furthermore, the mRNA activity of tissue-type plasminogen activator increases in pemphigus. Further studies are needed for more information about the pathogenicity of pemphigus in order to provide better treatments for this disease.

Conclusion

In general, the present study focused on reviewing all the available articles and investigating the role of RNAs in the pathophysiology of pemphigus. There is no certain target therapy for this disease. Nevertheless, considering that RNAs have a role in its pathophysiology and knowing that the effects of RNA pathways may be helpful in diagnosing and controlling the disease. The current study further evaluated the role of Mir-338-3p, miR-424-5p, MiR-127, miR-584-5p, and some mRNAs in pemphigus, and the results demonstrated that some RNAs may be impressive

Table 1. Summary of Presented Data in This Article

Type of RNAs	Type of disease	Changes	Mechanism	Year	References
mir-338-3p	PV	Increase	Causes an imbalance of Th1/Th2 cells thus it plays a role in the production of Dsg3 antibody	2018	(18, 19)
miR-424-5p	Pemphigus	Increase	Regulates p38 MAPK activation and HSP27 phosphorylation, and blister formation	2017	(4)
miR-127*	Pemphigus	-	Regulates the IL-6 expression	2017	(6)
miR-584-5p	Pemphigus Foliaceus	Increase	Regulates KLRG1 mRNA levels	2016	(28)
C3 mRNA	PV	Increase	IL-1a and TNF-a induce C3 synthesis	1999	(31, 36)
mRNA of CD36	PV	Increased both in isolated blood T cells and the patients' tissues	Regulates the immune response	2018	(32)
mRNA of CD163	PV	Increased only in the tissue	Regulates the immune response	2018	(32)
mRNA of tissue-type plasminogen activator	Pemphigus	Activity increase	May be related to regenerative attempts by the tissue	1990	(33)
mRNA of urokinase plasminogen activator	PV	Increase	IL-1a and TNF-a increase uPA expression and uPA is involved in acantholysis	2003	(39)
IL23R mRNA and RORyt mRNA	Pemphigus Foliaceus	Increase	Induction of RORyt expression depends on the expression of IL23R, and IL23 is necessary for maintenance and the proliferation of Th17 cells	2018	(41)
HLA-G1	PV	Increase	HLA-G has a role in reducing inflammation	2008	(44)
HLA-G2	PV	Decrease	HLA-G plays a role in reducing inflammation	2008	(44)
LAIR2 mRNA	Pemphigus	Higher levels of LAIR2 Mrna might influence PF susceptibility	Higher expression of LAIR2 in healthy controls.	2016	(27)

Note. LAIR2: Leukocyte-associated immunoglobulin-like receptor 2; PV: Pemphigus Vulgaris; PF: Pemphigus foliaceus; HLA: Human leukocyte antigen; TNF: Tumor necrosis factor-alpha; IL: Interleukin; uPA: Urokinase plasminogen activator receptor; KLRG1: Killer cell lectin like receptor G1. This article is controversial because the topic is related to miR-127 although miR-217 was mentioned in all parts of the article. It is believed that miR-217 is correct.

on pemphigus. However, more studies and clinical assessments are required for obtaining more information about the role of RNAs in pemphigus to get a better view of their mechanisms and use them as biomarkers for earlier diagnosis or maybe for treatment.

Ethics Approval

This study was approved by the Ethics Committee of Hamadan University of Medical Sciences (Ethical approval No. IR.UMSHA.REC.1399.126).

Availability of Data and Materials

The applied and/or analyzed datasets during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

Funding

The study was financially supported by the Vice-chancellor of Research and Technology, Hamadan University of Medical Sciences, Hamadan, Iran (Grant No. 9902231098).

Authors' Contributions

The conception and design of the study: F.N. and M.T. Acquisition of data, analysis, and interpretation of data: A.B. and F.N. The article drafting: A.B, M.S., and F.N. Critical revision of the article for important intellectual contents: MT, PH, MS, and FN. Final approval of the version for submission: MT and FN.

Acknowledgments

The authors would like to acknowledge the Vice-chancellor of Research and Technology, Hamadan University of Medical Sciences, Hamadan, Iran.

References

- Schmidt E, Kasperkiewicz M, Joly P. Pemphigus. Lancet. 2019;394(10201):882-94. doi: [10.1016/s0140-6736\(19\)31778-7](https://doi.org/10.1016/s0140-6736(19)31778-7).
- Walter E, Vielmuth F, Wanuske MT, Seifert M, Pollmann R, Eming R, et al. Role of Dsg1- and Dsg3-mediated signaling in pemphigus autoantibody-induced loss of keratinocyte cohesion. Front Immunol. 2019;10:1128. doi: [10.3389/fimmu.2019.01128](https://doi.org/10.3389/fimmu.2019.01128).
- Kitajima Y. Regulation and impairments of dynamic desmosome and corneodesmosome remodeling. Eur J Dermatol. 2013. doi: [10.1684/ejd.2013.1976](https://doi.org/10.1684/ejd.2013.1976).
- Wang M, Liang L, Li L, Han K, Li Q, Peng Y, et al. Increased miR-424-5p expression in peripheral blood mononuclear cells from patients with pemphigus. Mol Med Rep. 2017;15(6):3479-84. doi: [10.3892/mmr.2017.6422](https://doi.org/10.3892/mmr.2017.6422).
- Bystryń JC, Rudolph JL. Pemphigus. Lancet. 2005;366(9479):61-73. doi: [10.1016/s0140-6736\(05\)66829-8](https://doi.org/10.1016/s0140-6736(05)66829-8).
- Zhang Q, He Y, Cai W, Nie MH. MiR-127 regulates the development of oral pemphigus by targeting IL-6. Int J Clin Exp Med. 2017;10(4):6461-7.
- Grünwald D, Singer RH, Rout M. Nuclear export dynamics of RNA-protein complexes. Nature. 2011;475(7356):333-41. doi: [10.1038/nature10318](https://doi.org/10.1038/nature10318).
- Ertem G, Ferris JP. Synthesis of RNA oligomers on

- heterogeneous templates. *Nature*. 1996;379(6562):238-40. doi: [10.1038/379238a0](https://doi.org/10.1038/379238a0).
9. Small EM, Olson EN. Pervasive roles of microRNAs in cardiovascular biology. *Nature*. 2011;469(7330):336-42. doi: [10.1038/nature09783](https://doi.org/10.1038/nature09783).
 10. Olena AF, Patton JG. Genomic organization of microRNAs. *J Cell Physiol*. 2010;222(3):540-5. doi: [10.1002/jcp.21993](https://doi.org/10.1002/jcp.21993).
 11. Chitwood DH, Timmermans MC. Small RNAs are on the move. *Nature*. 2010;467(7314):415-9. doi: [10.1038/nature09351](https://doi.org/10.1038/nature09351).
 12. Bektas M, Jolly PS, Berkowitz P, Amagai M, Rubenstein DS. A pathophysiologic role for epidermal growth factor receptor in pemphigus acantholysis. *J Biol Chem*. 2013;288(13):9447-56. doi: [10.1074/jbc.M112.438010](https://doi.org/10.1074/jbc.M112.438010).
 13. Dhanapal R, Somasundarapandian S, Wihaskoro S, Kannan R, Rajkumar G, Chidambaram R. Interference RNA in immune-mediated oral diseases - minireview. *Cent Eur J Immunol*. 2017;42(3):301-4. doi: [10.5114/ceji.2017.70974](https://doi.org/10.5114/ceji.2017.70974).
 14. Salvatore M, Magrelli A, Taruscio D. The role of microRNAs in the biology of rare diseases. *Int J Mol Sci*. 2011;12(10):6733-42. doi: [10.3390/ijms12106733](https://doi.org/10.3390/ijms12106733).
 15. Abbott A. Rare-disease project has global ambitions. *Nature*. 2011;472(7341):17. doi: [10.1038/472017a](https://doi.org/10.1038/472017a).
 16. Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer*. 2006;6(11):857-66. doi: [10.1038/nrc1997](https://doi.org/10.1038/nrc1997).
 17. Negrini M, Nicoloso MS, Calin GA. MicroRNAs and cancer--new paradigms in molecular oncology. *Curr Opin Cell Biol*. 2009;21(3):470-9. doi: [10.1016/j.ceb.2009.03.002](https://doi.org/10.1016/j.ceb.2009.03.002).
 18. Liu Q, Cui F, Wang M, Xiong H, Peng X, Liang L, et al. Increased expression of microRNA-338-3p contributes to production of Dsg3 antibody in pemphigus vulgaris patients. *Mol Med Rep*. 2018;18(1):550-6. doi: [10.3892/mmr.2018.8934](https://doi.org/10.3892/mmr.2018.8934).
 19. Lin N, Liu Q, Wang M, Wang Q, Zeng K. Usefulness of miRNA-338-3p in the diagnosis of pemphigus and its correlation with disease severity. *PeerJ*. 2018;6:e5388. doi: [10.7717/peerj.5388](https://doi.org/10.7717/peerj.5388).
 20. Wan Y, Qu K, Zhang QC, Flynn RA, Manor O, Ouyang Z, et al. Landscape and variation of RNA secondary structure across the human transcriptome. *Nature*. 2014;505(7485):706-9. doi: [10.1038/nature12946](https://doi.org/10.1038/nature12946).
 21. Lobo-Alves SC, Augusto DG, Magalhães WCS, Tarazona-Santos E, Lima-Costa MF, Barreto ML, et al. Long noncoding RNA polymorphisms influence susceptibility to endemic pemphigus foliaceus. *Br J Dermatol*. 2019;181(2):324-31. doi: [10.1111/bjd.17640](https://doi.org/10.1111/bjd.17640).
 22. Xue J, Su W, Chen Z, Ke Y, Du X, Zhou Q. Overexpression of interleukin-23 and interleukin-17 in the lesion of pemphigus vulgaris: a preliminary study. *Mediators Inflamm*. 2014;2014:463928. doi: [10.1155/2014/463928](https://doi.org/10.1155/2014/463928).
 23. Jordon RE. Complement activation in pemphigus. *J Invest Dermatol*. 1980;74(5):357-8. doi: [10.1111/1523-1747.ep12543739](https://doi.org/10.1111/1523-1747.ep12543739).
 24. Son M, Santiago-Schwarz F, Al-Abed Y, Diamond B. C1q limits dendritic cell differentiation and activation by engaging LAIR-1. *Proc Natl Acad Sci U S A*. 2012;109(46):E3160-7. doi: [10.1073/pnas.1212753109](https://doi.org/10.1073/pnas.1212753109).
 25. Amagai M, Ahmed AR, Kitajima Y, Bystryk JC, Milner Y, Gniadecki R, et al. Are desmoglein autoantibodies essential for the immunopathogenesis of pemphigus vulgaris, or just "witnesses of disease"? *Exp Dermatol*. 2006;15(10):815-31. doi: [10.1111/j.1600-0625.2006.00499_1.x](https://doi.org/10.1111/j.1600-0625.2006.00499_1.x).
 26. Lebbink RJ, de Ruiter T, Adelmeijer J, Brenkman AB, van Helvoort JM, Koch M, et al. Collagens are functional, high affinity ligands for the inhibitory immune receptor LAIR-1. *J Exp Med*. 2006;203(6):1419-25. doi: [10.1084/jem.20052554](https://doi.org/10.1084/jem.20052554).
 27. Camargo CM, Augusto DG, Petzl-Erler ML. Differential gene expression levels might explain association of LAIR2 polymorphisms with pemphigus. *Hum Genet*. 2016;135(2):233-44. doi: [10.1007/s00439-015-1626-6](https://doi.org/10.1007/s00439-015-1626-6).
 28. Cipolla GA, Park JK, de Oliveira LA, Lobo-Alves SC, de Almeida RC, Farias TD, et al. A 3'UTR polymorphism marks differential KLRG1 mRNA levels through disruption of a miR-584-5p binding site and associates with pemphigus foliaceus susceptibility. *Biochim Biophys Acta*. 2016;1859(10):1306-13. doi: [10.1016/j.bbagr.2016.07.006](https://doi.org/10.1016/j.bbagr.2016.07.006).
 29. de Almeida RC, Chagas VS, Augusto DG, Magalhães WCS, Tarazona-Santos E, Castro MAA, et al. Integrative Genome-Wide Analysis Identify Variants Located at MicroRNA Binding Sites Associated with Pemphigus Foliaceus. *HLA*. 2017;89(6):370.
 30. Völlner F, Ali J, Kurrle N, Exner Y, Eming R, Hertl M, et al. Loss of flotillin expression results in weakened desmosomal adhesion and pemphigus vulgaris-like localisation of desmoglein-3 in human keratinocytes. *Sci Rep*. 2016;6:28820. doi: [10.1038/srep28820](https://doi.org/10.1038/srep28820).
 31. Feliciani C, Toto P, Amerio P. In vitro C3 mRNA expression in pemphigus vulgaris: complement activation is increased by IL-1alpha and TNF-alpha. *J Cutan Med Surg*. 1999;3(3):140-4. doi: [10.1177/120347549900300306](https://doi.org/10.1177/120347549900300306).
 32. Das D, Anand V, Khandpur S, Sharma VK, Sharma A. T helper type 1 polarizing $\gamma\delta$ T cells and Scavenger receptors contribute to the pathogenesis of pemphigus vulgaris. *Immunology*. 2018;153(1):97-104. doi: [10.1111/imm.12814](https://doi.org/10.1111/imm.12814).
 33. Baird J, Lazarus GS, Belin D, Vassalli JD, Busso N, Gubler P, et al. mRNA for tissue-type plasminogen activator is present in lesional epidermis from patients with psoriasis, pemphigus, or bullous pemphigoid, but is not detected in normal epidermis. *J Invest Dermatol*. 1990;95(5):548-52. doi: [10.1111/1523-1747.ep12504901](https://doi.org/10.1111/1523-1747.ep12504901).
 34. Ameglio F, D'Auria L, Bonifati C, Ferraro C, Mastroianni A, Giacalone B. Cytokine pattern in blister fluid and serum of patients with bullous pemphigoid: relationships with disease intensity. *Br J Dermatol*. 1998;138(4):611-4. doi: [10.1046/j.1365-2133.1998.02169.x](https://doi.org/10.1046/j.1365-2133.1998.02169.x).
 35. D'Auria L, Bonifati C, Mussi A, D'Agosto G, De Simone C, Giacalone B, et al. Cytokines in the sera of patients with pemphigus vulgaris: interleukin-6 and tumor necrosis factor-alpha levels are significantly increased as compared to healthy subjects and correlate with disease activity. *Eur Cytokine Netw*. 1997;8(4):383-7.
 36. Feliciani C, Toto P, Amerio P, Pour SM, Coscione G, Shivji G, et al. In vitro and in vivo expression of interleukin-1alpha and tumor necrosis factor-alpha mRNA in pemphigus vulgaris: interleukin-1alpha and tumor necrosis factor-alpha are involved in acantholysis. *J Invest Dermatol*. 2000;114(1):71-7. doi: [10.1046/j.1523-1747.2000.00835.x](https://doi.org/10.1046/j.1523-1747.2000.00835.x).
 37. Anhalt GJ, Patel HP, Labib RS, Diaz LA, Proud D. Dexamethasone inhibits plasminogen activator activity

- in experimental pemphigus in vivo but does not block acantholysis. *J Immunol.* 1986;136(1):113-7.
38. Schuh T, Besch R, Braungart E, Flaig MJ, Douwes K, Sander CA, et al. Protease inhibitors prevent plasminogen-mediated, but not pemphigus vulgaris-induced, acantholysis in human epidermis. *Biol Chem.* 2003;384(2):311-5. doi: [10.1515/bc.2003.035](https://doi.org/10.1515/bc.2003.035).
 39. Feliciani C, Toto P, Wang B, Sauder DN, Amerio P, Tulli A. Urokinase plasminogen activator mRNA is induced by IL-1alpha and TNF-alpha in in vitro acantholysis. *Exp Dermatol.* 2003;12(4):466-71. doi: [10.1034/j.1600-0625.2002.120415.x](https://doi.org/10.1034/j.1600-0625.2002.120415.x).
 40. Arakawa M, Dainichi T, Yasumoto S, Hashimoto T. Lesional Th17 cells in pemphigus vulgaris and pemphigus foliaceus. *J Dermatol Sci.* 2009;53(3):228-31. doi: [10.1016/j.jdermsci.2008.09.008](https://doi.org/10.1016/j.jdermsci.2008.09.008).
 41. Ben Jmaa M, Abida O, Fakhfakh R, Bahloul E, Sellami K, Gaddour L, et al. Involvement of the IL23/Th17 pathway in the pathogenesis of Tunisian pemphigus foliaceus. *Mediators Inflamm.* 2018;2018:8206983. doi: [10.1155/2018/8206983](https://doi.org/10.1155/2018/8206983).
 42. Carosella ED, Moreau P, Le Maoult J, Le Discorde M, Dausset J, Rouas-Freiss N. HLA-G molecules: from maternal-fetal tolerance to tissue acceptance. *Adv Immunol.* 2003;81:199-252. doi: [10.1016/s0065-2776\(03\)81006-4](https://doi.org/10.1016/s0065-2776(03)81006-4).
 43. Arnold B, Schönrich G, Ferber I, Alferink J, Hämmerling GJ. Peripheral T cell tolerance: distinct levels and multistep mechanisms. In: Bluethmann H, Ohashi PS, eds. *Transgenesis and Targeted Mutagenesis in Immunology*. Boston: Academic Press; 1994. p. 135-48. doi: [10.1016/b978-0-12-105760-2.50012-1](https://doi.org/10.1016/b978-0-12-105760-2.50012-1).
 44. Yari F, Zavaran Hosseini A, Nemat Gorgani M, Khorramizadeh MR, Mansouri P, Kazemnejad A. Expression of HLA-G in the skin of patients with pemphigus vulgaris. *Iran J Allergy Asthma Immunol.* 2008;7(1):7-12.