Granzyme B: A novel therapeutic target for treatment of atopic dermatitis

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Abstract

Granzyme B is a serine protease that can play multiple roles in intracellular and extracellular perforin-dependent or non-perforin-dependent mechanisms. Granzyme B has been found to be an important factor involved in the pathogenesis of atopic dermatitis and is increased in both skin lesions and peripheral blood of atopic dermatitis patients. In this article, we review the correlation between granzyme B and atopic dermatitis to provide a novel therapeutic targeting option for clinical treatment of the latter.

Key words: Granzyme B, atopic dermatitis, pathogenesis, treatment

Introduction

Granzyme B, a multifunctional serine protease thought to be derived from cytotoxic T lymphocytes and natural killer cells, is secreted and expressed along with perforin for the induction of apoptosis.[1] Indeed, granzyme B also plays an important role in the extracellular non-perforin-dependent environment of many inflammatory diseases, including some lung diseases, cardiovascular diseases and skin diseases, promoting inflammatory responses, influencing extracellular matrix remodelling and inducing epithelial-mesenchymal transition and fibrosis.[2] Atopic dermatitis is a chronic inflammatory skin disease characterised by pruritus and skin lesions and is governed by a variety of genetic, environmental and immunological factors. Epidemiological surveys show that atopic dermatitis affects 5–20% of children and 2–10% of adults worldwide, and the prevalence is increasing.[3] The pathogenesis of atopic dermatitis is still not fully understood, and most of the existing treatments have associated side effects. Several recent studies have demonstrated that granzyme B is detected at elevated levels in skin lesions and peripheral blood of atopic dermatitis patients and is involved in the disruption of atopic dermatitis barrier function, the development of inflammatory responses, increased vascular permeability and several other potential pathogenic mechanisms. In this review, we discuss the pathogenic mechanisms of granzyme B in atopic dermatitis, which promises to be a new therapeutic target for atopic dermatitis.

Granzyme B

In the 1980s Masson *et al.*[4] identified two serine proteases in cytotoxic T lymphocytes cells, one of which, a 29-kDa protein consisting of a single polypeptide chain, was named granzyme B. For a long time granzyme B was thought to be expressed only by cytotoxic T lymphocytes and natural killer cells, and only since the turn of the century has granzyme B been found to be expressed in a variety of non-immune cells such as smooth muscle cells, keratinocytes and chondrocytes under certain conditions.[5] Therefore, granzyme B has now been shown to function via two diverse pathways. One is a classical perforin-dependent apoptosis-inducing function, and the other is a non-apoptotic function independent of perforin, which is closely related to the pathogenesis of atopic dermatitis. Gapud *et al.*[6] treated human submandibular gland cell cultures with granzyme B in the absence of perforin, and although cleavage of intracellular proteins was still present, no significant induction of death was

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detected by flow cytometry after 90 minutes, and nuclear morphological changes of apoptosis were similarly not observed by immunofluorescence microscopy. This suggests that granzyme B may perform other potential functions in the presence of perforin deficiency. It has been found that approximately one-third of granzyme B escapes from the immune synapse to the extracellular environment during entry into the target cell. Since the extracellular environment lacks effective granzyme B inhibitors, it can remain active there and then cleave a variety of substrates (e.g., cytokines, proteins, enzymes), resulting in a range of pro-inflammatory responses including protein cleavage and epithelial barrier disruption, fibrosis, inflammation and autoimmunity.[7] For example, in autoimmune blistering diseases, granzyme B degrades anchoring proteins, induces inflammatory factor secretion and neutrophil infiltration; in cutaneous leishmaniasis, it promotes the production of tumour necrosis factor and interleukin-6 and correlates positively with the size of lesions in the disease; in pemphigus, it cleaves several key proteins and leads to blister formation.[8–10] Therefore, granzyme B may be found as a key inducer and/or modulator in a variety of inflammation-related skin diseases.[11]

The role of Granzyme B in the pathogenesis of atopic dermatitis

Yawalkar *et al.*[12] observed significantly higher expression of granzyme B in skin lesions than in normal skin in eight patients with moderate to severe atopic dermatitis. Kamata *et al.*[13] also found by enzyme immunoassay that plasma granzyme B concentrations in atopic dermatitis patients were elevated and positively correlated with indicators of atopic dermatitis severity, and that plasma granzyme B levels were significantly lower after treatment with some conventional drugs compared to the untreated group. These experimental results suggest that granzyme B may play a crucial role during atopic dermatitis. The onset and development of atopic dermatitis are multifactorial, with the following three being the main pathways currently identified: firstly, impaired skin barrier function, secondly, T helper-mediated cellular immune imbalance, and thirdly, reduced skin microbial diversity characterised by an increased proportion of *Staphylococcus aureus* colonisation.[14,15] It is important to clarify that these mechanisms do not cause the disease independently but interact with each other, leading to recurrent episodes and a chronic course of atopic dermatitis. To date, research has found that granzyme B is primarily involved in the pathogenesis of the first two aspects of atopic dermatitis, which are discussed in detail below. A brief summary is presented in Table 1.

Disruption of the skin barrier

Turner *et al.*[16] administered a granzyme B inhibitor (VTI-1002) topically to the skin lesion area of an oxazolone-induced atopic dermatitis mouse model and after a period of time detected a reduction in the loss of filaggrin and E-cadherin proteins in atopic dermatitis skin compared to controls. By comparing measurements of transepidermal water loss in granzyme B knockout oxazolone mice and wild-type oxazolone mice, the results showed that transepidermal water loss was significantly lower in the former compared to the latter, all of which suggest a disruptive effect of granzyme B on the skin barrier.[16] In addition, it has been suggested that granzyme B also cleaves fibronectin and laminin, which are abundant at the dermo-epidermal junction, and that degradation of these proteins may potentially compromise skin integrity, reduce nutrient exchange and decrease the stability of the dermo-epidermal junction.[17] On the other hand, Merkulova *et al.*[18] demonstrated using HaCaT cell lines in vitro that granzyme B also delayed the migration of keratinocytes by interfering with the epidermal growth factor receptor pathway, thereby inhibiting the normal healing of damaged skin. It is worth noting that the increased expression of granzyme B in skin keratinocytes under ultraviolet irradiation accelerates the degradation of collagen, the main component of the dermis, and promotes the onset of photoaging and consequent weakening of the barrier function of the skin.[19]

Promotes inflammatory response

In 2010, Omoto *et al.*[20] demonstrated that granzyme B cleaves the inactive precursor interleukin-18 from 24-kDa to an 18-kDa fragment between Aspartic 35- tyrosine 36 and that the cleaved fragment has the same structural sequence and biological activity as the caspase-1 enzyme at the interleukin-18 cleavage site. The following year, Afonina *et al.*[21] reported that granzyme B also cleaved interleukin-1α at the Aspartic 103 site, which in turn significantly increased the pro-inflammatory activity of interleukin-1α. Previous studies have demonstrated that both interleukin-18 and interleukin-1α drive the development of chronic skin inflammation in atopic dermatitis. Interleukin-18 induces interferon-γ secretion, promotes interleukin-4 expression and enhances mast cell infiltration, which is strongly correlated with the clinical severity of atopic dermatitis patients, whereas epidermal microbial dysbiosis and barrier damage in atopic dermatitis stimulate keratinocytes to

Table 1: A summary of the role of granzyme B in atopic dermatitis

Disruption of the skin barrier	
Loss of filaggrin and E-cadherin protein	Increased TEWL
Reduced dermal-epidermal cohesion	Decreased stability of DEJ
Delayed keratinocyte migration	Skin healing slows down
Accelerated degradation of collagen	Promotes skin photoaging
Promotes inflammatory response	
Enhance the activity of inflammatory factors	IL-18, IL-1α
Promote the release of inflammatory cytokines	MIP-2/ IL-8, IL-1β
Enhance vascular permeability	
Promote the release of VEGF	Increased vascular permeability causing plasma exudation
Others	
Promote the release of GRP	Develop pruritus
Activation of Th2-associated immune cells	Induce allergic reaction

TEWL: Transepidermal water loss, DEJ: Dermal-epidermal junction, IL: Interleukin, MIP-2: Macrophage inflammatory protein-2, VEGF: Vascular endothelial growth factor, GRP: Gastrin-releasing peptide,Th2: T helper 2.

release abnormal amounts of interleukin-1α inducing an increased inflammatory response.[22,23] Recently, a mouse model of pemphigoid demonstrated that granzyme B also promotes macrophage inflammatory protein-2 in a dose-dependent manner, and interestingly, granzyme B showed the same effect on interleukin-8, a homolog of human macrophage inflammatory protein-2, which is a key factor mediating neutrophil chemotaxis.[8] Hu *et al.*[24] added 15 nM granzyme B to human colon adenocarcinoma (HCT-8) cells medium and found a significant increase in interleukin-1β levels by polymerase chain reaction after 6 hours. Further comparison of the medium with and without lipopolysaccharide showed that granzyme B enhanced the lipopolysaccharide-induced inflammatory response in HCT-8 cells.[24] Although the mechanism by which granzyme B enhances the secretion of many of these inflammatory factors is still not well understood, the role of granzyme B in promoting or inducing inflammatory responses is undeniable.

Enhancement of vascular permeability

Hendel *et al.*[25] found that granzyme B increased the amount of vascular endothelial growth factor in the extracellular matrix by promoting the separation of fibronectin from vascular endothelial growth factor conjugates and that the separated vascular endothelial growth factor was not only biologically active but also induced further phosphorylation of vascular endothelial growth factor receptor 2, thus causing increased plasma leakage due to enhanced vascular permeability. Shen *et al.*[26] used angiotensin-converting enzyme II to construct a mouse model of cardiac fibrosis and observed reduced permeability of cardiac microvessels and reduced plasma leakage in granzyme B knockout mice compared to wild controls, further confirming the possible role of granzyme B in enhancing vascular permeability. A study has shown that vascular endothelial growth factor, a potent vascular permeability factor and pro-pathogenic angiogenic factor, is significantly elevated in the stratum corneum of atopic dermatitis patients and correlates positively with the severity of their lesions.[27] We therefore speculate that granzyme B may be involved in the development of atopic dermatitis.

Other actions

Plasma levels of gastrin-releasing peptide (a pruritus-related peptide) and granzyme B were also found to be positively correlated in atopic dermatitis patients, so granzyme B may also mediate the onset of pruritus, the main symptom of atopic dermatitis.[13] Asthma, which is also an atopic disease, is a common co-morbidity of atopic dermatitis and there is a significant correlation with atopic dermatitis, with elevated serum immunoglobulin E levels being observed in the course of both.[28] Qian *et al.*[29] demonstrated that granzyme B expressed by natural killer cells activates pulmonary group 2 innate lymphoid cells and T helper cells by stimulating interlukin-25 production in airway epithelial cells, thereby inducing an eosinophilic inflammatory response in the airways leading to the development of allergic airway disease. This leads us to speculate that granzyme B may play a similar role in mediating atopic dermatitis-specific allergic responses, but the evidence for direct induction of atopic dermatitis immune responses by granzyme B is currently insufficient and needs to be confirmed by additional studies in the future.

Perspectives on Granzyme B for atopic dermatitis

Based on basic research into the mechanism of action of granzyme B in atopic dermatitis, more and more researchers are realising that the treatment of atopic dermatitis by inhibiting granzyme B activity is very promising clinically. Serine protease inhibitors (serpins) work by using their reaction centre ring as a pseudo-substrate and cleavage site for homologous proteases, forming covalent complexes with the corresponding proteases to render them biologically inactive.[30] SerpinB9 is a natural inhibitor of intracellular granzyme B, which unfortunately has not yet been found in the extracellular environment. Subsequently, Saito *et al.*[31] transplanted OVA-specific CD8+ T (OT-I) cells (transgenic $CD8^+$ T cells expressing ovalbumin-specific T-cell receptor) into chicken ovalbumin transgenic mice to construct a mossy tissue reaction/interface dermatitis model and after five days of treatment with serpina3n, a murine extracellular serinease inhibitor, observed not only a significant improvement in the symptoms of skin lesions compared to the control group but also pathological findings showing a reduction in the number of OT-I cell infiltrates. Also, the onset of acute graft-versus-host disease was delayed after prophylactic administration of serpina3n to OT-I cell receptor ovalbumin mice.[31] However, although this granzyme B inhibitor found in mouse plasma is homologous to human α1-antichymotrypsin, the latter is mainly expressed in the liver and whether they have the same function and mechanism of action has not been clarified.[32]

The recent development of a highly selective and potent human granzyme B small molecule inhibitor in Vancouver, namely: VTI-1002, has largely advanced the progress of drug research based on granzyme B as a therapeutic target.[33] Shen *et al.*[34] applied VTI–1002 gel to the skin lesions of burn model mice continuously for a total of 30 days, resulting in faster wound healing and less scar formation in the VTI–1002 gel–treated mice compared to the excipient–only control group. It is important to highlight that the concentration of VTI–1002 in the skin remained virtually unchanged over 4–24 hours, suggesting that it can remain at the application site for a long enough time to be effective.[34] Further studies by Turner *et al.*[16] on the oxazolone-induced atopic dermatitis mouse model exhibiting thinning of ear thickness and reduced signs of skin lesions such as scaling, erythema and erosions following the application of VTI-1002 gel demonstrated that VTI-1002 works by blocking or reducing granzyme B-mediated loss of filaggrin and E-cadherin proteins to improve skin barrier function. So far, although the studies on granzyme B inhibitors are still in the experimental stage in animal models, they have all shown significant therapeutic effects and no adverse effects, which provides a theoretical basis and data reference for future clinical trials.

Conclusions

In summary, granzyme B is an important protein molecule involved in the pathogenesis of atopic dermatitis, and therapies targeting granzyme B have undoubtedly opened new research ideas for its management. However, the specific regulatory mechanisms of granzyme B in atopic dermatitis are not well understood, and whether inhibition of granzyme B expression and reducing its level can improve the inflammatory status and skin barrier function in atopic dermatitis patients, and whether it can be a biomarker for monitoring changes in the disease course and assessing prognosis remains to be investigated in more depth and observed in long-term clinical trials.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Conflict of interest

There are no conflicts of interest.

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