GRANZYME B MEDIATES DEGRADATION OF HEMIDESMOSOME PROTEINS IN STEVENS-JOHNSON SYNDROME/TOXIC EPIDERMAL NECROLYSIS

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Abstract

- Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe blistering skin reactions triggered by an adverse response to certain medications.
- Elevated Granzyme B (GzmB) levels were observed in skin samples from SJS/TEN patients, along with reduced levels of skin proteins that anchor the layers (epidermis and dermis) together.
- We also detected elevated GzmB and fragments of important skin proteins in blister fluids from patients. This suggests that GzmB contributes to the degradation these anchoring proteins in SJS/TEN.
- Experimentally, we showed that GzmB cuts these skin proteins.
- These findings provide new insights into the conditions and may pave the way for innovative treatments.

Background

- GzmB is a serine protease historically known for its role in immune cell-mediated apoptosis.
- During prolonged and/or dysregulated inflammation, GzmB can accumulate extracellularly where it can cleave extracellular matrix, basement membrane, cell junction and cell surface receptors [1].
- GzmB is elevated in autoimmune blistering diseases (bullous pemphigoid(BP), epidermolysis bullosa acquisita(EBA)) and accumulates at the dermal-epidermal junction (DEJ) [1][2].
- Knockout or topical inhibition of GzmB reduces degradation of DEJ proteins and blistering in murine models of BP/EBA [2].

Granzyme B Mechanism

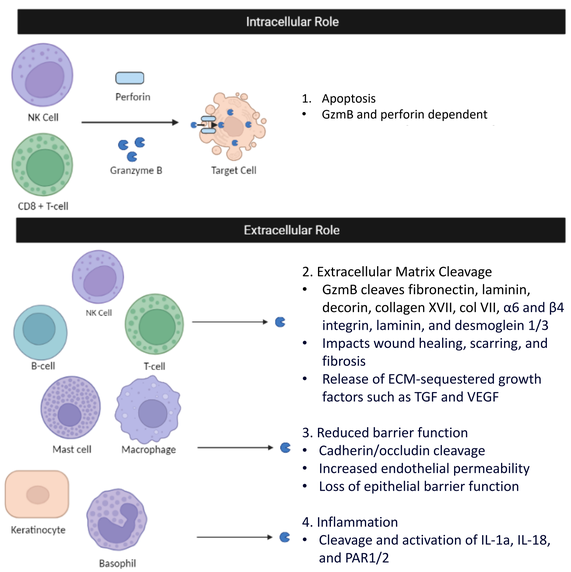

Figure 1. Intracellular and extracellular roles of GzmB. Modified from [3] in BioRender.

Research Hypothesis

- While GzmB is elevated in several different autoimmune blistering conditions, its role and mechanism has not been fully investigated in the context of SJS/TEN.
- In the present study, we ***hypothesize*** that GzmB mediates degradation of dermal-epidermal junction proteins in the spectrum of SJS/TEN by contributing to epidermal detachment and blistering.

Methodology

Immunohistochemistry:

- Human skin biopsies from 8 SJS/TEN patients were sectioned, incubated with anti-GzmB, anti-collagen XVII, anti-integrin α6 and anti-integrin β4 antibodies and imaged with Novared staining.

Western blot:

- α6 and β4 integrin sub-units, and Collagen XVII were incubated in 200nM GzmB at 37˚C for 24hrs.
- Prior to initiation of cleavage assay, GzmB inhibitors Compound 20 (100μM) or Serpin A3N (600μM) were incubated with 200nM of GzmB for 1hr at 37˚C.
- Immunodetection of Collagen XVII NC16A domain of SJS/TEN, TEN, Bullous Pemphigoid, and Linear IgA bullous disease blister fluid (adverse drug reaction related condition).

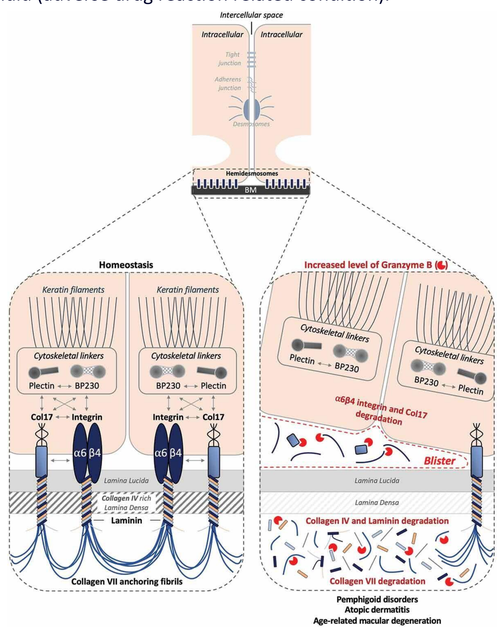

Figure 2. GzmB mediated dermal-epidermal dysfunction through basement membrane degradation and hemidesmosome dissolution. [4]

Results

GzmB is elevated at the DEJ in blisters of SJS/TEN patients compared to normal skin.

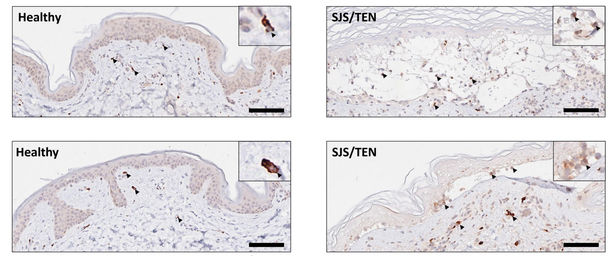

Figure 3. GzmB IHC of healthy (N=8) and SJS/TEN skin biopsies (N=8). Elevated GzmB positive cells are present at the level of the DEJ. Scale bars represent 100μM.

β4 integrin is cleaved by GzmB in vitro. Reduced β4 integrin is observed in SJS/TEN patient skin compared to healthy skin.

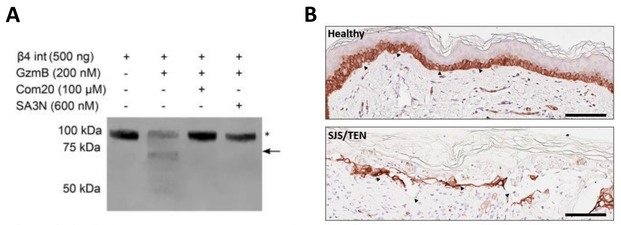

Figure 4. (A) β4 integrin cleavage assay with and without GzmB inhibitors Compound 20 (Com20) and Serpin A3N (SA3N) [1]. β4 integrin subunits were incubated with 200nM GzmB at 37˚C for 24 hours with or without prior incubation of Com20 (100μM) or SA3N (600μM). (B) β4 integrin immunostaining of healthy (N=8) and SJS/TEN skin biopsies (N=8). Scale bars represent 100μM

Collagen XVII is cleaved by GzmB in vitro. Reduced collagen XVII is observed in SJS/TEN patient skin compared to healthy skin and collagen XVII fragments are detected in human blister fluid.

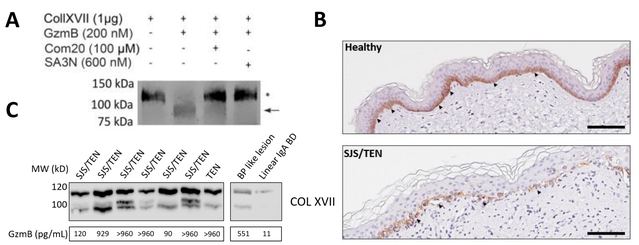

Figure 5. (A) Collagen XVII cleavage assay with and without GzmB inhibitors Compound 20 (Com20) and Serpin A3N (SA3N) [1]. Recombinant Collagen XVII was incubated with 200nM GzmB at 37˚C for 24 hours with or without prior incubation with Com20 (100μM) or SA3N (600μM). (B) Collagen XVII immunostaining of healthy (N=8) and SJS/TEN skin biopsies (N=8). Scale bars represent 100μM. (C) Immunodetection of Collagen XVII NC16A domain in SJS/TEN, TEN, BP like lesion and Linear IgA Bullous Disease blister fluid. (SDS-PAGE; 7%). Quantification of GzmB in blister fluid was done by ELISA.

α6 integrin is cleaved by GzmB in vitro. Reduced α6 integrin is observed in SJS/TEN patient skin compared to healthy skin.

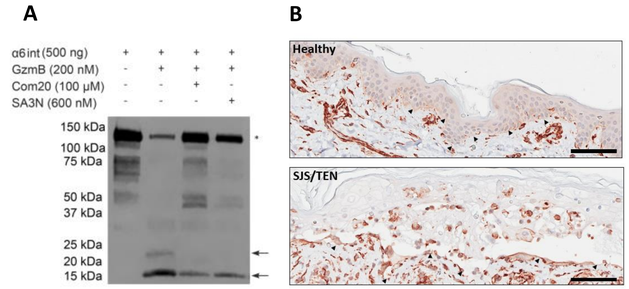

Figure 6. (A) α6 integrin cleavage assay with and without GzmB inhibitors Compound 20 (Com20) and Serpin A3N (SA3N) [2]. Recombinant α6 integrin was incubated with 200nM GzmB at 37˚C for 24 hours with or without prior incubation with Com20 (100μM) or SA3N (600μM). (B) α6 integrin immunostaining of healthy (N=8) and SJS/TEN skin biopsies (N=8). Scale bars represent 100μM.

Conclusions

- GzmB is present in SJS/TEN blister fluids and is observed proximal to DEJ separation in SJS/TEN skin.
- GzmB cleaves key DEJ proteins that anchor the epidermis to the dermis.
- Cleaved collagen XVII fragments are found in SJS/TEN Blister fluid around 97-100kDa.
- Reduced Collagen XVII, β4 integrin, and α6 integrin in SJS/TEN skin may be attributed to increased GzmB proteolytic activity.

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