further investigations are necessary which are now possible because
of the non-invasive procedure of the method. Nevertheless, this pilot
study has shown that in future studies, potential influences of body
regions on the SAAID must be considered.

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uted the 2PM device, JL, MCM, MED designed the research study, CC
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CONFLICT OF INTEREST

The authors state no conflict of interest.

Granzyme A potentiates chemokine production in
IL-17-stimulated keratinocytes

Abstract

Plaque psoriasis presents with focal skin inflammation, partially
maintained by IL-17-mediated interactions between infiltrating epi-
dermal T cells and activated keratinocytes. Here we show that the
majority of lesional epidermal CD8 T cells express granzyme A, alone
or in combination with IL-17. To assess proinflammatory properties
of granzyme A in psoriasis, primary human keratinocytes were stimu-
lated with granzyme A in the presence or absence of IL-17. Out of 33
analysed keratinocyte-derived inflammatory mediators, granzyme A
potentiates IL-17-induced secretion of CXCL 1, CXCL 12 and CCL 4.

Keywords

autofluorescence, SAAID, second harmonic generation, wide-field
2PM

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Intriguingly, all three chemokines are implicated in psoriasis pathogenesis and are involved in recruitment of T cells, neutrophils and pDCs into inflamed tissues. Our results indicate that granzyme A produced by lesional CD8 T cells specifically increase the chemokine production from inflamed keratinocytes, thereby amplifying a chemotactic inflammatory loop that sustains psoriasis lesions.

1 | BACKGROUND

Granzymes (Gzms), a family of granule serine proteases that is stored in cytotoxic lymphocytes, have the capacity to induce cellular cytotoxicity in the presence of perforin.\(^1\) In psoriasis, where massive T-cell infiltration occurs in lesional skin,\(^2,3\) gene expression of GZMA and GZMB is upregulated in lesional skin alongside with genes related to the Th17/IL-23 axis.\(^4,5\) We have previously reported elevated gene expression of GZMA, GZMB and PRF (perforin) in CD8 T cells sorted from psoriasis lesions as compared to healthy skin.\(^6\) Despite increased frequency of GzmB- and perforin-expressing cells in lesional psoriasis compared to normal skin, atopic (AD) and allergic contact dermatitis (ACD) lesions harbour significantly more granzyme-expressing T cells as compared to psoriasis (s1).\(^7\) Moreover, apoptotic cells are scarce in psoriasis (s3) and keratinocytes from psoriasis lesions show resistance to apoptosis (s2), indicating alternative functionality of Gzms in psoriasis compared to AD and ACD. Non-cytolytic activity of Gzms is reported, and in particular, GzmA induces expression of proinflammatory cytokines IL-1β, IL-6 or IL-8 in primary monocytes,\(^8\) fibroblasts or epithelial cell lines (s4). Interestingly, GzmA-deficient mice display no defects in cellular cytotoxicity but impaired LPS-\(^8\) or bacterial-induced septic shock,\(^9\) further supporting its proinflammatory role (s4).

2 | QUESTIONS ADDRESSED

We postulated that granzymes act as a proinflammatory mediator in psoriasis. Granzyme and perforin expression in CD8 T cells from psoriasis lesions was assessed and primary keratinocytes were stimulated with GzmA and IL-17 to assess secretion of inflammatory mediators.

3 | EXPERIMENTAL DESIGN

See Supporting Information.

4 | RESULTS

4.1 | Granzyme A-expressing CD8 T cells accumulate in psoriasis lesions

Intracellular expression of GzmA, GzmB and perforin was assessed in T cells extracted from active psoriasis lesions and healthy skin (Figure 1). A small proportion of GzmB+ or perforin+ lesional CD8 T cells (Figure 1A) indicated limited Gzm-mediated cellular cytotoxicity in psoriasis in accordance with earlier studies (s2, s3). In contrast, the majority of CD8 T cells from psoriasis lesions expressed GzmA. Confocal microscopy confirmed intracellular GzmA+ granules in epidermal T cells (Figure 1B,C).

4.2 | Granzyme A potentiates chemokine production in IL-17-stimulated keratinocytes

In psoriasis, CD8 T cells producing IL-17A accumulate in epidermis\(^6\) (s6) in close contact with keratinocytes that respond to IL-17 stimulation with production of innate cytokines and chemokines.\(^3\) In the limited number of patients included in this study, no correlation between the frequency of GzmA+ T cells and lesional leucocyte infiltration or disease severity could be determined (data not shown). However, epidermal GzmA-expressing CD8 T cells produced the psoriasis-associated cytokines IFN-γ and IL-17A upon stimulation (Figure 1D,E). To investigate whether GzmA potentiates psoriasis-related cytokine or chemokine production, primary human keratinocytes derived from healthy skin were incubated with endotoxin-free GzmA (Fig. S1) in subcytotoxic concentration\(^10\) (s5) in the presence or absence of IL-17A.
GzmA neither promoted proliferation nor affected the IL-17-induced keratinocyte differentiation status as measured by gene expression of MKI67, IVL, KRT16 or KRT10 (Fig. S2A). A multiplex immunoassay was used to screen supernatants for secretion of 33 different inflammatory mediators (Table, Supporting information). IL-17 induced secretion of the cytokines IL-6, IL-8 and IL-23 (Figure 2, Table, Supporting information), whereas GzmA treatment alone did not induce secretion of inflammatory mediators. Interestingly, the combination of GzmA and IL-17 significantly increased the release of the proinflammatory chemokines CXCL1, CXCL12, and CCL4 compared to IL-17 stimulation alone, but did not alter the levels of IL-17-induced cytokines IL-6, IL-8 and IL-23 (Figure 2, Table, Supporting information). Upregulation of CXCL1 in IL-17-stimulated keratinocytes by GzmA was blocked by the serine protease inhibitor 3,4-dichloroisocoumarin (DCI) (Fig. S2B). IL-1α secretion was increased by GzmA alone or in combination with IL-17 as previously reported,[9] but this difference did not reach statistical significance.

CONCLUSIONS

Proinflammatory activity of GzmA[8] has not been explored in the context of skin diseases. Here, we show that CD8 T cells in psoriasis skin lesions display a dominant expression of GzmA over GzmB in the absence of perforin expression. Signals recruiting GzmA+ T cells into the skin and triggers of GzmA expression in the context of chronic inflammation deserve future investigation and potentially Tc17-polarizing conditions favour development of GzmA+GzmB−Prf− CD8 T cells in the context of psoriasis. We found that GzmA potentiated IL-17-induced secretion of three of the 33 inflammatory mediators analysed in keratinocytes. Intriguingly, all three chemokines, CXCL1, CXCL12 and CCL4, are involved in recruitment of T cells, neutrophils and pDCs into inflamed tissues and have been implicated in psoriasis (s7-s9).

Our results indicate that GzmA produced by lesional CD8 T cells has the capacity to specifically increase chemokine secretion from inflamed keratinocytes, thereby sustaining the focal inflammation in psoriasis lesions by amplifying a chemotactic inflammatory loop. GzmA may additionally potentiate the effect of other proinflammatory mediators on keratinocytes or stromal cells present in the skin. Given the involvement of CD8 T cells in various inflammatory skin diseases (s10-s12), the potential proinflammatory function of Gzms in other skin diseases warrant further investigation.

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CONFLICT OF INTEREST

The authors have declared no conflicting interests.

AUTHOR CONTRIBUTION

SC, EM, KB, DC and BR performed the experiments. SC and LE designed the research study. BR, MS and LE contributed essential reagents or tools. SC, EM and LE analysed the data. SC and LE wrote the manuscript that was read and revised by all authors.

ETHICAL APPROVAL

The study was performed according to the Declaration of Helsinki principles and approved by the Stockholm Regional Committee of Ethics (approval number 2012/50-31/2). Written consent was collected from all donors.

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Keywords
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FIGURE 2 Granzyme A potentiates chemokine secretion by IL-17-treated keratinocytes. Concentration of cytokine or chemokine (CXCL1, CXCL12, CCL4, IL-1α, IL-23, IL-8) secreted from keratinocytes treated with IL-17A (10 ng/mL) or kept in medium, with or without the presence of granzyme A (35 nM), Mean±SD from four donors is depicted. RM one-way ANOVA, Turkey’s multiple comparisons tests. *P<.05; ns: not significant (P>.05). Refer to the Table (Supporting information) for the complete list.
Niacinamide leave-on formulation provides long-lasting protection against bacteria in vivo

Abstract
Antimicrobial peptides (AMPs) form a part of the skin’s innate immune system. Their primary activity is to provide antimicrobial benefits and hence protect from infections. AMPs that are present on human skin include psoriasin (S100A7), RNase 7, lysozyme, LL-37 and defensins. Niacinamide is a well-known cosmetic ingredient that has been used traditionally for skin lightening, anti-ageing and skin barrier building benefits.[3,4] Recent data indicate that niacinamide treatment can boost AMPs in gut epithelial cells and in neutrophils. Treatment with niacinamide in mice also provided protection from skin infections by enhancing AMPs. In this article, we find that treatment with niacinamide formulation provides long-lasting protection against bacteria, potentially through the activation of an AMP response.

1 | BACKGROUND
Skin is an immunologically active organ and harbours immune mediators from the innate and adaptive branches of immunity. Skin innate immunity is critical for cutaneous defense and antimicrobial peptides (AMPs) form a part of the skin’s innate immune system. Primary activity of the AMPs is to provide antimicrobial benefits and protect from infections.[1,2]

Niacinamide is a well-known cosmetic ingredient that has been used traditionally for skin lightening, anti-ageing and skin barrier building benefits.[3,4] Recent data indicate that niacinamide treatment can boost AMPs in gut epithelial cells and in neutrophils.[5,6] Treatment with niacinamide in mice also provided protection from infections through the boost of AMPs.[6] Based on these studies, we hypothesized that niacinamide can elicit similar responses in human skin resulting in enhanced antimicrobial protection and boosting AMP in skin cells.

2 | QUESTION ADDRESSED
To evaluate the effect of a niacinamide containing formulation in providing antimicrobial benefits to human skin.

3 | EXPERIMENTAL DESIGN
Supplementary section Data S1.