A CARD10-Dependent Tonic Signalosome Activates MALT1 Paracaspase and Regulates IL-17/TNF-α–Driven Keratinocyte Inflammation


TO THE EDITOR

The paracaspase MALT1 controls signaling downstream of several cell surface receptors, such as C-type lectin receptors on myeloid cells and antigen receptors on lymphocytes. Upon receptor engagement, MALT1, BCL10, and a CARD family member assemble into a “CBM complex,” which is required to trigger MALT1 paracaspase activity and downstream transcriptional activation mechanisms (Meininger and Krappmann, 2016; Rosebeck et al., 2011). Here, we found that CARD10 is highly expressed in proliferating keratinocytes and is responsible for a tonic level of paracaspase activity, driven by MALT1 isoform A. Furthermore, using the potent and selective MALT1 inhibitor MLT-827 (Bardet et al., 2018; Unterreiner et al., 2017), we show that MALT1 activity regulates proinflammatory responses downstream of IL-17/TNF-α.

The physiological pathways leading to MALT1 activation in keratinocytes are just starting to be explored. It was recently reported that MALT1 can be activated by dectin-1, a C-type lectin receptor, whereby it contributes to keratinocyte inflammation (Schmitt et al., 2016). Here, we investigated the role of MALT1 in normal human epidermal keratinocytes (NHEKs) stimulated by IL-17/TNF-α, a synergistic proinflammatory combination known to recapitulate key features of psoriasis (Chiricozzi et al., 2011; Hedrick et al., 2009). We first tested the effect of MLT-827 on CXCL8 (IL-8 mRNA) and IL-8 levels in NHEKs challenged with

Abbreviation: NHEK, normal human epidermal keratinocyte

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IL-17A/TNF-α. CXCL8 induction was sensitive to inhibition by MLT-827 (Figure 1a) and, consistently, IL-8 production under these conditions was down-modulated by MLT-827 with a half maximal inhibitory concentration of 48 nmol/L (Figure 1b). In addition to CXCL8, we measured IL17C, the transcript encoding the keratinocyte-enriched IL-17 family member IL-17C (Johnston et al., 2013). IL17C induction upon stimulation with IL-17A/TNF-α was strongly reduced by MLT-827 (Figure 1c). Other responsive genes, such as ZC3H12C (encoding regnase-1) and NFKBIZ were not sensitive to MLT-827 (see Supplementary Figure S1a online). Up-regulation of IL-8 downstream of IL-17C/TNF-α was also reduced by MLT-827, with a half maximal inhibitory concentration of 19 nmol/L (Figure 1d). Altogether, by contributing to the signaling of IL-17A and IL-17C, MALT1 appears poised to control an important mechanism for amplification of keratinocyte inflammation.

Intriguingly, the proinflammatory combination of IL-17A and TNF-α, although sensitive to MALT1 protease inhibition, was, in contrast to phorbol 12-myristate 13-acetate/ionomycin, unable to increase MALT1 proteolytic function, which was monitored by following the cleavage of several known MALT1 substrates, such as RelB, CYLD, and HOIL-1 (Jaworski and Thome, 2016; Klein et al., 2015) (Figure 1e). However, we noticed significant basal levels of paracaspase activity in unchallenged NHEKs, which contrasts with the complete absence of MALT1 activity in naïve immune cells (Coornaert et al., 2008; Meininger et al., 2016). This was also the case in other epithelial cells, such as A549 lung adenocarcinoma cells (see Supplementary Figure S1b). The basal level of MALT1 activity was sensitive to inhibition by the pan-PKC inhibitor AEB071 (Figure 1e). This suggested that a PKC-dependent recruitment of MALT1 into CBM complexes, which represents the canonical MALT1 activation pathway, might occur in proliferating keratinocytes.

A recent report about MALT1A and MALT1B, which encode two functionally distinct isoforms, describes that primary naïve lymphocytes express exclusively MALT1B and up-regulate MALT1A in response to activation (Meininger et al., 2016). Compared with MALT1B, MALT1A displays an additional binding site for recruitment of TRAF6, allowing for stronger scaffolding and signaling properties (Ginster et al., 2017; Meininger et al.,...
In contrast to naïve lymphocytes, we found that MALT1A is the major isoform expressed by NHEKs (Figure 2a), which could potentially explain the elevated basal MALT1 activity level observed in proliferating keratinocytes.

Keratinocytes are known to express CARD14, for which several gain-of-function mutations have recently been reported as segregating with PSOR2 (Akiyama, 2016; Jordan et al., 2012). In addition to CARD14, we found that proliferating NHEKs express CARD10 (Figure 2b, and see Supplementary Figure S2a online). Because NHEKs are able to differentiate in response to calcium or when establishing cell-cell contacts (Hennings et al., 1980) (see Supplementary Figure S2b), we plated both post-confluent and sub-confluent...
NHEKs and followed their expression of CARD10 and CARD14 upon calcium- or confluency-induced differentiation. Absolute quantification measurements showed that CARD10 levels are 10-fold higher than CARD14 levels in proliferating NHEKs. During differentiation, CARD10 was up-regulated and CARD10 was down-regulated, resulting in both CARDS reaching similar levels by day 3 (Figure 2b, and see Supplementary Figure S2c). These data strongly suggest that CARD10 might play an underappreciated role in keratinocytes, particularly during their proliferation.

Next, we investigated whether CARD10 contributes to the tonic MALT1 activity described (Figure 1e). Knocking down MALT1 or CARD10 by CRISPR/Cas9 (Figure 2c and 2d) abrogated RelB cleavage, showing that CARD10 is essential for the constitutive level of MALT1 activity in proliferating NHEKs. We also monitored the cleavage of A20, a known substrate of MALT1 in lymphocytes (Coornaert et al., 2008), which was independently shown to regulate IL-17R signaling in keratinocytes (Garg et al., 2013). As with RelB, A20 cleavage was abrogated in MALT1-knockout and CARD10-knockout cells (Figure 2c). Previous work has shown that CARD10 and MALT1 can signal downstream of EGFR (liang et al., 2011; Pan et al., 2015). We thus hypothesized that growth factors might be involved in the up-regulation of MALT1 activity under proliferative conditions. When the growth factor containing supplement was removed (starved condition), RelB cleavage was drastically reduced compared with the complete medium condition. RelB cleavage was also reduced when cells in complete medium were treated with a combination of EGFR and IGF-R inhibitors (gefitinib and AEW541) (Figure 2e) (García-Echeverría et al., 2004; Prahlad et al., 2012). Collectively, these results illustrate that growth factors contribute to basal MALT1 paracaspase activity in proliferative keratinocytes through a CARD10-dependent mechanism.

Finally, to address how MALT1 regulates IL-17A/TNF-α-driven inflammation, we compared various settings to influence the basal level of MALT1 activity. IL-8 production in response to IL-17A/TNF-α significantly increased in complete medium, an effect that was abrogated with the gefitinib/AEW541 combination. The increase of IL-8 production in complete medium was fully blocked by MLT-827, indicating that growth factors contribute to IL-17/A/TNF-α proinflammatory signaling via stimulation of MALT1 paracaspase activity (Figure 2f). When EGFR signaling was kept to a minimum, in basal medium or in the presence of the gefitinib/AEW541 combination, IL-8 was produced at low levels. However, these low levels remained sensitive to MLT-827, indicating that MALT1 can influence the IL-17/A/TNF-α proinflammatory pathway by additional mechanisms.

In conclusion, by using a potent and selective MALT1 inhibitor, we provide evidence that MALT1 proteolytic function regulates the IL-17/TNF-α pathway in keratinocytes. The mechanism involves a CARD10-dependent pathway, likely through CBM complex formation between CARD10, BCL10, and MALT1A. In addition, MALT1 may influence IL-17A/TNF-α signaling by other means. The MALT1A isoform strongly binds to TRAF6, which itself is a key component of the IL-17R signaling cascade (Amaty et al., 2017). Whether TRAF6 acts as a bridge for connecting a CBM signalsome to the IL-17R pathway or whether TRAF6 independently recruits MALT1 to the IL-17R pathway remains to be explored (Israel and Bornancin, 2018). Several known protein partners of MALT1 (e.g., TRAF6, A20, or regnase-1) are reported to interfere with IL-17 signaling. Furthermore, keratinocyte-specific MALT1 substrates remain to be identified, which might contribute to the regulation of the IL-17A/TNF-α pathway by MALT1.

CONFLICT OF INTEREST
The authors are employees of Novartis and many own Novartis stock. The authors state no conflict of interest related to this work.

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SUPPLEMENTARY MATERIAL
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REFERENCES
Blockade of Granzyme B Remarkably Improves Mucocutaneous Diseases with Keratinocyte Death in Interface Dermatitis


TO THE EDITOR

Lichenoid tissue reaction/interface dermatitis (LTR/IFD) is a pattern of skin inflammation characterized pathologically by infiltration of the skin basement membrane by auto-aggressive T cells, causing keratinocyte death (Sontheimer and Gilliam, 1981). A wide spectrum of skin disorders exhibit this set of histological features and mucocutaneous lesions, including acute graft-versus-host disease (aGVHD). Previous clinical studies on disorders with LTR/IFD have described that CD8⁺ T cells are the most important cells that infiltrate the lesion. The infiltrating CD8⁺ T cells were distributed around apoptotic keratinocytes and express PRF1 and granzyme (Gzm) B (Correia et al., 2001; Jungell et al., 1989; Paller et al., 1988; Takata et al., 1993). Serum levels of soluble FasL produced by CD8⁺ T cells were also reportedly increased in patients with aGVHD (Liem et al., 1998). Studies with allogeneic bone marrow transplant murine models also supported these clinical observations (Baker et al., 1996; Braun et al., 1996; Graubert et al., 1996). We particularly evaluated the impact of PRF1/Gzm and FasL/Fas pathways on LTR/IFD development.

We used chicken ovalbumin (OVA) transgenic mice, in which keratinocytes express membrane-bound OVA under the control of a keratin 14 promoter (K14-mOVA mice). K14-mOVA mice develop aGVHD-like mucocutaneous disease (Supplementary Figure S1 online) and weight loss was observed after transfer of transgenic CD8⁺ T cells expressing OVA-specific T-cell receptor (from GFP transgenic OT-I mice) (Shibaki et al., 2004). Weight loss during late-phase reflects the severity of mucocutaneous lesions causing difficulty in food intake. Hematoxylin and eosin staining of ear specimens showed LTR/IFD (Supplementary Figure S2 online). Immunofluorescence staining with anti-GFP antibodies revealed infiltration of GFP⁺ OT-I cells in the epidermis and dermis, which resulted in a number of dead keratinocytes revealed by TUNEL assay (Supplementary Figure S2). The disease is mediated by CD8⁺ T cells (Supplementary Figure S3 online).

To investigate the roles of cytotoxic molecules on LTR/IFD development, we observed wild-type (WT), PRF1−/−, GzmA−/−, GzmB−/−, and FasL−/− OT-I cell–transferred recipient K14-mOVA mice. PRF1−/− OT-I cell– and GzmB−/− OT-I cell recipients did not develop mucocutaneous lesions, whereas GzmA−/− OT-I cell recipients developed milder lesions than those in WT OT-I cell recipients at 14 days post-OT-I cell transfer (Figure 1a, 1b). PRF1−/− OT-I cell recipients and GzmB−/− OT-I cell recipients completely recovered their body weights, whereas GzmA−/− OT-I cell recipients presented a late-phase weight loss similar to WT (Figure 1c). Hematoxylin and eosin–stained ear specimens of PRF1−/− OT-I and GzmB−/− OT-I cell recipients did not show LTR/IFD without dead keratinocytes and mild infiltration.

Abbreviations: aGVHD, acute graft-versus-host disease; Gzm, granzyme; LTR/IFD, lichenoid tissue reaction/interface dermatitis; OVA, ovalbumin; WT, wild-type

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