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SHORT COMMUNICATION

Leukotrienes Do Not Modulate the Course of Aldara™-induced Psoriasiform Dermatitis in Mice

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Leukotrienes are a group of lipid mediators exerting multiple physiological and pathophysiological functions (1). Leukotriene B_4 (LTB_4) often critically regulates immune cell recruitment to sites of inflammation (2, 3), and LTC_4 enhances vascular permeability and induces tissue fibrosis in inflammation (1). In line with this, LTB_4 has been shown to initiate the recruitment of neutrophils and T cells into the skin in atopic dermatitis, while LTC_4 regulates the development of dermal fibrosis in this disease (4). In contrast, the role of leukotrienes in psoriasis is still elusive, although LTB_4 and LTC_4 are both highly elevated in psoriatic plaques (5, 6) and epicutaneous application of LTB_4 onto human skin induces skin alterations resembling psoriatic lesions (7–9).

Results of clinical trials addressing the efficiency of leukotriene inhibition to treat psoriasis have however been inconclusive with some trials showing significant clinical improvement (10, 11) and others finding no significant effects (12).

In 2009, a mouse model for psoriasis based on the application of Aldara™ cream was described (13). We will refer to this model as "Aldara-induced psoriasiform dermatitis" (AIPD). Imiquimod is the active compound in Aldara and activates TLR7/8. Additionally, the vehicle in Aldara is pharmacologically active and activates the inflammasome. Both activities of the cream are required to achieve full-blown dermatitis in the AIPD model (14).

We set out to clarify the significance of leukotrienes in the pathogenesis of the AIPD model.

MATERIALS AND METHODS

Alox5$^{-/-}$ and *Ltb4r1*$^{-/-}$ mice on the C57BL/6 background were purchased from The Jackson Laboratory, wild-type mice from Charles River. All lines were bred in the animal facility of the University of Lübeck. Mice were used for experiments when 8–10 weeks old. The local government had approved all animal experiments.

To induce AIPD, a 2 × 3 cm area on the back was shaved and depilated on day –2 of the experiment. Starting on day 0, 50 mg Aldara™ cream (Meda, Solna, Sweden), containing 5% imiquimod, were applied daily on this area for 5 consecutive days. Dermatitis was evaluated using a modified Psoriasis Activity and Severity Index (PASI). Briefly, erythema, infiltration, and desquamation were individually scored on a scale from 0–4 with 0, none; 1, mild; 2, moderate; 3, marked; 4; severe. The scores of these individual aspects of dermatitis were added to obtain a composite score.

For histopathology, skin biopsies obtained from lesional skin on day 6 were fixed in formalin, paraffin-embedded, and H&E stained. The epidermal thickness was determined by measuring the distance between the derma–epidermal junction and the epidermal surface using ImageJ software. Sections were stained for Ki-67 using rat anti-murine Ki-67, biotinylated goat anti-rat IgG (Biolegend), and DyLight 488-streptavidin (Thermoscientific). The number of Ki-67$^+$ keratinocytes/μm^2 in the epidermis was quantified by BZ-II Analyzer software (Keyence), as previously described (15). All data are presented as mean ± SEM. Clinical scores were analysed by two-way-ANOVA with Bonferroni posttest, epidermal thickness by one-way ANOVA with Tukey's posttest. $p < 0.05$ was considered statistically significant. All calculations were performed with GraphPad Prism 5.0 software.

RESULTS

To determine the contribution of leukotrienes to the pathogenesis of psoriasis, we examined the development of psoriasis-like skin lesions in the AIPD mouse model, comparing disease in *C57BL/6* wild-type mice vs. 5-lipoxygenase gene-deficient (*Alox5*$^{-/-}$) mice. The key enzyme in the biosynthesis of all leukotrienes is 5-lipoxygenase. By day 3 of Aldara application, both wild-type and *Alox5*$^{-/-}$ mice developed signs of psoriasiform skin inflammation, which further progressed and peaked with pronounced erythema, infiltration, and desquamation (Fig. S1A[1]).

On day 6, lesional wild-type and *Alox5*$^{-/-}$ mice both exhibited pronounced skin infiltration by myeloid and lymphoid cells as well as epidermal hyperproliferation. These histopathological parameters were indistinguishable between the 2 groups (Fig. S1C–F[1]).

We also examined AIPD in BLT1-deficient (*Ltb4r1*$^{-/-}$) mice. BLT1 is the only high-affinity receptor of LTB_4 and largely responsible for its effects in inflammation. Comparing the clinical course of AIPD in wild-type vs. *Ltb4r1*$^{-/-}$ mice, we found no significant contribution of BLT1-mediated effects of LTB_4 to skin inflammation (data not shown). Aldara induced severe skin inflammation in both groups of mice, excluding a significant role of BLT1 signalling in this model.

DISCUSSION

Leukotrienes have been implicated in the pathogenesis of psoriasis since they were found highly expressed

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in lesional skin (5, 6). However, due to inconclusive clinical trials addressing the therapeutic efficiency of leukotriene inhibition in psoriasis patients and due to the lack of suitable mouse models of psoriasis, the significance of leukotriene effects for the pathogenesis of psoriasis remained uncertain (10–12).

Elucidating the pathogenesis of psoriasis has always been particularly challenging due to the limitations of existing mouse models, which are predominantly based on instigating intrinsic imbalances of skin homeostasis and rarely reflect inflammation as driver of the pathogenesis of psoriasis. We have seized the opportunity to address the significance of leukotriene effects in the pathogenesis of psoriasis provided by the emergence of the new AIPD mouse model (13). Using *Alox5*$^{-/-}$ mice, which cannot biosynthesize leukotrienes, we did not find any contribution of leukotrienes to the course of psoriasiform skin inflammation. Likewise, deficiency in BLT1, the high-affinity receptor of LTB_4, did not affect the course of skin inflammation. These findings are in stark contrast to findings made in several other models of organ-specific inflammation, including atopic dermatitis, in which LTB_4 and its receptor BLT1 were required to initiate effector cell recruitment (4).

As a corollary, the mechanisms of effector cell recruitment into the skin must be stimulus-dependent. The pathways activated by Aldara application to recruit effector cells into the skin are either completely independent of leukotrienes or, alternatively, there are other overlapping pathways providing redundancy in effector cell recruitment into the skin, thus fully compensating for the loss of leukotrienes. The fact that leukotrienes are highly elevated in human skin and topical application of LTB_4 mimics skin alterations typical for psoriatic skin lesions argues for the latter hypothesis.

The proinflammatory stimuli provided by Aldara cream in AIPD are quite potent and simultaneously activate TLR7 and inflammasome signalling, while inhibiting the anti-inflammatory effect exerted via endogenous adenosine receptors. As a result, several major proinflammatory pathways are activated in parallel and consequently induce a broad panel of proinflammatory chemokines and cytokines in the skin, creating optimal conditions for the emergence of severe skin inflammation, which may override the contribution of factors usually required to initiate skin inflammation. We therefore cannot exclude that LTB_4/BLT1 may contribute noticeably to the induction of disease when the inciting stimuli are more moderate.

Our data suggest that leukotriene release is an epiphenomenon reflecting the activation of myeloid cells in psoriasiform dermatitis, or alternatively, leukotrienes are just one of several redundant pathways orchestrating psoriasiform skin inflammation. Further pursuing monotherapeutic strategies aiming at inhibiting leukotriene biosynthesis or leukotriene receptors to improve psoriasis treatment are therefore unlikely to be effective.

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The authors declare no conflict of interest.

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