IL-36 Signaling Is Essential for Psoriatic Inflammation through the Augmentation of Innate Immune Responses

TO THE EDITOR
IL-36 cytokines are composed of three agonists, namely IL-36α, IL-36β, and IL-36γ, and a natural antagonist, IL-36Ra (Sims and Smith, 2010). IL-36 cytokines are abundantly expressed by the skin and other epithelial tissues, whereas the IL-36 receptor (IL-36R) is expressed by skin and immune cells, including dendritic cells (DCs) (Gabay and Towne, 2015; Vigne et al., 2011). Earlier studies have demonstrated that IL-36 cytokines play important roles in the development of psoriasisiform inflammation by enhancing the function of T helper type 17 cytokines (Carrier et al., 2011; Tortola et al., 2012). Indeed, IL-36 signaling—regulated genes in keratinocytes (KCs) are largely shared with those observed in psoriatic lesions, and they form interconnected feedback loops that potentiate IL-17 signaling and leukocyte chemotaxis (Carrier et al., 2011; Mahil et al., 2017), although the details remain largely unknown.

Psoriatic lesions demonstrated increased IL-36α, IL-36γ, IL-36Ra, but not IL-36β, as reported previously (see Supplementary Figure S1a online). Similarly, all of the IL-36 family members were upregulated in 12-O-tetradecanoylphorbol-13-acetate (TPA)—induced psoriatic lesions in K5.Stat3C model (see Supplementary Figure S1b), which required the IL-23/Th17 pathway (Nakajima et al., 2011).

To explore the role of IL-36 signaling in KCs, we examined psoriasis-related gene expression in response to exogenous IL-36 in between control and IL-36R−/− (knockout [KO]) KCs derived from IL-36R KO mice. Following IL-36α stimulation, wild-type KCs expressed increased I36a, S100a8, Deltb3, and I17c mRNAs, and also IL-17C protein (Figure 1a). In contrast, the upregulation of those was not observed in IL-36R KO KCs. Similar results were obtained when we used other IL-36 cytokines, namely, IL-36β and IL-36γ (Supplementary Figure S2 online). These results strongly implicated the participation of the IL-36/IL-36R autocrine loop within KCs in psoriasis

Abbreviations: DC, dendritic cell; Ht, heterozygous; IL-36R, IL-36 receptor; KC, keratinocyte; KO, knockout; TPA, 12-O-tetradecanoylphorbol-13-acetate

Accepted manuscript published online 17 December 2018; corrected proof published online 8 March 2019
© 2018 The Authors. Published by Elsevier, Inc. on behalf of the Society for Investigative Dermatology.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-nd/4.0/
Figure 1. IL-36 signaling is required for both keratinocytes and dendritic cells to express psoriasis signature molecules to develop psoriasiform lesions. (a) Reverse transcriptase PCR and ELISA assay using keratinocytes from WT (n = 4–5) and IL-36R KO (KO, n = 3) mice. White, untreated; black, IL-36α–treated (100 ng/ml, 24 hours). **P < 0.01, ***P < 0.001, ****P < 0.0001. (b) Reverse transcriptase PCR analysis. Black bars, HKC (5 × 10⁶ cells/ml); white bars, untreated control. WT, n = 4–7; KO, n = 3–8. *P < 0.05. (c) Reverse transcriptase PCR. HKC (5 × 10⁶ cells/ml), IL-36α (100 ng/ml, 12 hours), n = 3–7, **P < 0.01 by one-way analysis of variance with Tukey-Kramer analysis. (d, e) ELISA assay using the culture supernatant of splenic dendritic cells. White bars,
development. Our recent study revealed that in vitro IL-36 stimulation of KCs elicited Stat3 phosphorylation at the early time point (Takaishi et al., 2018), suggesting that the IL-36/IL-36R autocrine loop may facilitate the Stat3-mediated innate immunity within KCs.

There are immunologic similarities between anti-Candida defense and psoriasis, including the IL-23/IL-17 axis (Saunte et al., 2017). Psoriasis signature molecules were upregulated in keratinocytes when treated in vitro with heat-killed Candida albicans, including IL-8 (Kobayashi et al., 2009; Pivarcsi et al., 2003). Interestingly, stimulation with heat-killed C. albicans resulted in the upregulation of IL36a, IL17c, and Ccl20 in wild-type KCs, but not in IL-36R KO KCs (Figure 1b). In addition, heat-killed C. albicans stimulation exerted an additive effect on IL-36 signaling in IL36a and Ccl20 expression in KCs (Figure 1c and Supplementary Figure S3 online). This result suggested that KCs could respond to C. albicans through pattern recognition receptors, leading to the transcriptional activation of IL36a, IL17c, and Ccl20 in a manner depending on the IL-36/IL-36R autocrine loop, which is shared with psoriasis and may involve the IL-23/T helper type 17 axis (Saunte et al., 2017). The role of IL-36 signaling in splenic DCs was clearly shown by the increase of IL-12/IL-23p40

untreated control, n = 4 (d, e); black bars, IL-36Ra-treated (1 µg/ml, 12 hours), n = 4 (d); IMQ-treated (20 µM, 12 hours), n = 4. **P < 0.01, ****P < 0.0001. (f) Histology of ear skin from K5.Stat3C:IL-36R<sup>+</sup> (top) and K5.Stat3C:IL-36RKO mice (bottom) at day 3 of topical TPA treatment. Hematoxylin and eosin staining. Scale bars = 200 µm. (g) Ear swelling. White bar, K5.Stat3C:IL-36R controls (n = 11); black bar, K5.Stat3C:IL-36RKO mice (n = 7). **P < 0.01. (h) Reverse transcriptase PCR analysis using ear skin. White bars, K5.Stat3C:IL-36R<sup>+/+</sup> mice (n = 10); black bars, K5.Stat3C:IL-36RKO mice (n = 6). *P < 0.05, **P < 0.01. (i, j) Ear swelling in chimeric K5.Stat3C mice over time (i, hours) and at 24 hours (j) of topical TPA treatment. Ht>Ht, IL-36R<sup>+/+/</sup> bone marrow cells (n = 4); Ht>KO, IL-36R<sup>+/−</sup> mice with IL-36R<sup>+/−</sup> bone marrow cells (n = 7); KO>Ht, IL-36R<sup>+/−</sup> mice with IL-36R<sup>−/−</sup> bone marrow cells (n = 5); KO>KO, IL-36R<sup>−/−</sup> mice with IL-36R<sup>−/−</sup> bone marrow cells (n = 5). *P < 0.05. (k) Reverse transcriptase PCR analysis using TPA-induced skin lesions of bone marrow chimeric mice of the indicated genotype combinations (n = 4–6 in each group) at 72 hours. *P < 0.05, **P < 0.01. HKC, heat-killed Candida albicans; Ht, heterozygous; IMQ, imiquimod; KO, knockout; ND, not detectable; ns, not significant; TPA, 12-O-tetradecanoylphorbol-13-acetate; WT, wild-type.
and tumor necrosis factor-α following stimulation with IL-36α. This DC activation was mediated by IL-36R because DCs from IL-36RKO mice are impaired in the production of these cytokines (Figure 1d). Our previous study demonstrated that a cathepsin K inhibitor, which damped toll-like receptor 7–mediated DCs activation leading to IL-23 production, attenuated TPA-induced psoriasiform development in K5.Stat3C mice, suggesting the role of innate immunity with DCs (Hirai et al., 2013). Notably, stimulation of toll-like receptor 7 with imiquimod resulted in an increase of tumor necrosis factor-α and IL-12/IL-23p40 production by control DCs, but not by IL-36R KO DCs (Figure 1e). This result highlights the role of IL-36 signaling to enhance the innate immune response through toll-like receptor 7 in DCs. We next sought to address the in vivo effect of IL-36R deficiency on psoriasiform inflammation in K5.Stat3C mice. Deficiency of IL-36R protected from TPA-induced skin lesions in K5.Stat3C mice (Figure 1f, g), while those with IL-36R expression on radio-resistant epidermis via IL-36 signaling (Figure 1a). However, IL36 mRNA levels were decreased in lesions of mice with both Ht>KO and KO>Ht chimeras (Figure 1k). Collectively, these results suggested that bona fide psoriasiform lesions depended on IL-36 signaling both in the epidermis and in bone marrow–derived cells, including DCs. In contrast, the previous study showed that IL-36R expression on radio-resistant skin-resident cells, but not on bone marrow–derived cells, was essential for pathology upon imiquimod treatment (Tortola et al., 2012). The discrepancy of the results in the present and previous studies might be due to the difference in mouse models, although the underlying mechanism remains unknown.

Given that psoriasis develops via IL-36 signaling, the inhibition of that pathway should attenuate psoriatic inflammation. To explore this possibility, we treated K5.Stat3C mice and ex vivo tissue cultures of human plaque psoriasis with recombinant IL-36Ra. Subcutaneous injection of IL-36Ra attenuated the development of TPA-induced skin lesions in K5.Stat3C mice (Figure 2a, b, and Supplementary Figure S6 online). In addition, the epithelial thickness of psoriasis lesions in ex vivo cultures was attenuated by the addition of IL-36Ra (Figure 2c, d), which also downregulated IL8, CCL20, and IL17A mRNAs (Figure 2e). In conclusion, these results strongly suggest that the inhibition of IL-36 signaling would be relevant for new treatments of psoriasis.

Supplementary Figure S7 online illustrates the role of IL-36 signaling (red jagged mark) both in KCs and in DCs in the augmentation of innate immune responses, which leads to the development of psoriasis through activation of the IL-23/IL-17 axis.

All experimental procedures performed on mice were approved by the Institutional Animal Care and Use Committee of the Kochi Medical School. The written informed consent and approval of the ethics committee were obtained from all patients.

ORCID: Kentar Ohko: http://orcid.org/0000-0002-7614-126X

CONFLICT OF INTEREST: The authors state no conflict of interest.

ACKNOWLEDGMENTS: We thank Reiko Kamijima for her help with the histopathology, and JE Sims, JE Towne, and Amgen Inc for providing us with IL-36RKO mice. This work was supported in part by a Grant-in-Aid for Scientific Research (26461695) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Kentar Ohko1, Kimiko Nakajima1, Sayo Kataoka1,2, Mikiro Takaishi1 and Shigetoshi Sano1,2,*

1Department of Dermatology, Kochi Medical School, Kochi University, Nankoku, Japan; and 2Science Research Center, Kochi University, Nankoku, Japan

*Corresponding author e-mail: sano.derm@kochi-u.ac.jp

SUPPLEMENTARY MATERIAL: Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.12.003.


A Western Diet, but Not a High-Fat and Low-Sugar Diet, Predisposes Mice to Enhanced Susceptibility to Imiquimod-Induced Psoriasiform Dermatitis


TO THE EDITOR
Psoriasis is a disease with systemic inflammation and accompanied by multiple comorbidities, including metabolic syndrome and obesity (Hwang et al., 2017; Tsai et al., 2017; Yu et al., 2017). Obesity is often observed in patients with psoriasis and may precede development of psoriasis (Debbaneh et al., 2014). Western diet (WD) plays a crucial role in the development of obesity in Western countries and is characterized by elevated amounts of fat and sugars, especially simple sugars such as sucrose (Jena et al., 2017). Recent research has revealed that, in a murine model, WD triggers systemic inflammation via the NLRP3 inflammasome and subsequent production of pro-inflammatory cytokines, such as IL-1β (Christ et al., 2018).

On the other hand, a high-fat diet (HFD) without excessive sugars is often used to establish murine models of obesity. Herein, we established two models of obesity by feeding mice with a WD or HFD. The WD is not only rich in fat but also has a high sucrose content, replicating the type of diet in the Western world that contributes to obesity. The HFD obtains 60% of calories from fat and has a sucrose content that is similar to regular mouse chow. We compared these two kinds of diet-induced obese mice with lean mice on control diets in terms of susceptibility to imiquimod (IMQ)-induced psoriasiform dermatitis (PsD). Animal protocols were approved by the Institutional Animal Care and Use Committee at the University of California, Davis. Of interest, mice on HFD gained more weight than mice on WD by week 7 of feeding and sustained this increased body mass over the course of the experiment (Figure 1a, b). The measurement of ear thickness change following exposure to topical IMQ (Cochez et al., 2017; Yu et al., 2019) or intradermal IL-23 (Mabuchi et al., 2011) is a standard method to assess the extent of PsD. Although mice fed with HFD had the greatest weight gain, mice fed with WD for 12 or 16 weeks had significantly more ear thickness change than mice fed with control diet, HFD, and low-fat diet after a 5-day IMQ treatment course (P < 0.0001) (Figure 1c, d). There was no difference in ear thickness change between HFD- and low-fat diet–fed mice on day 5. Thus, mice on WD, but not HFD, had enhanced susceptibility to IMQ-induced PsD 3–4 months after sustained feeding, despite greater weight gain in HFD-fed mice. WD-fed mice showed consistently greater epidermal hyperplasia than the other three dietary groups (Figure 1e, f). Because neutrophils are a characteristic feature of psoriatic lesions and may play a key pathogenic role in psoriasis, we quantified neutrophil abscess formation as well as neutrophil chemoattractants in the four dietary groups. While there was no difference in Cxcl1 expression, WD-fed mice had higher expression of Cxcl2 than HFD-fed mice after 5-day IMQ treatment (Figure 1g, h). WD-fed mice had the highest expression level of neutrophil marker Ly6g mRNA and the highest density of Munro micro-abscess (Figure 1i, j). Consistent with reverse transcriptase PCR findings, more Gr-1(+) cells were observed in IMQ-treated WD-fed mice than other groups (Figure 1k, l). Expression levels of Ly6c mRNA were not different among four dietary groups, which indicates higher expression of Gr-1 in WD-fed mice results from higher expression of Ly6g but not Ly6c (Supplementary Figure 1 online).

Therefore, compared to the other three dietary groups, WD-fed mice had increased epidermal hyperplasia in response to IMQ, as well enhanced expression of a neutrophil chemo-attractant, which may explain the histologic appearance of IMQ-treated skin in the WD-fed mice. Our previous research has indicated CCR6+ T helper type 17 cells and γδ T cells secrete IL-17A in murine psoriasiform models (Mabuchi et al., 2013). Because IL-17A is a key mediator of neutrophilic inflammatory states, we postulated that IL-17A may help explain the enhanced neutrophilic infiltration observed in WD-fed mice after IMQ treatment. As anticipated, IMQ induced higher gene expression of IL-17a in all four dietary groups (Figure 2a). To determine whether the different diets could alter baseline expression of inflammatory cytokines in skin, we next assessed their expression in vehicle-treated ears, which reflect expression of these cytokines in the absence of IMQ stimulation. Strikingly, WD induced a 45-fold increase of IL-17a compared with control diet, while HFD induced only a 20-fold increase (WD vs. HFD).

Abbreviations: HFD, high-fat diet; IMQ, imiquimod; PsD, psoriasiform dermatitis; WD, Western diet

Received 17 December 2018; accepted in revised form 8 March 2019

© 2018 The Authors. Published by Elsevier, Inc. on behalf of the Society for Investigative Dermatology.