HHS Public Access

Author manuscript

Allergy. Author manuscript; available in PMC 2021 August 01.

Published in final edited form as:

Allergy. 2020 August ; 75(8): . doi:10.1111/all.14260.

Interleukin-36 (IL-36) system in the 1-fluoro-2,4-dinitrobenzene (DNFB) mouse model of allergic contact dermatitis

Angela Zaladonis, **Xinyan Zhang**, **Katherine K. Manupipatpong**, **Sowmiya Kalaiselvan**, **Pilar Alvarez**, **Liselotte E. Jensen**

Department of Microbiology and Immunology, Temple University Lewis Katz School of Medicine, Philadelphia, PA, USA

To the Editor,

Inflammatory processes are critical for protective immunity against microbes, but can lead to allergies and autoinflammatory diseases when excessive. Consequently, anti-inflammatory mechanisms have evolved to restrain these responses. A well-characterized example of a complete self-regulated inflammatory pathway is the interleukin-1 (IL-1) system comprising the agonist cytokines IL-1α and IL-1β, and their receptor IL-1R1 (IL-1 receptor type I), which is inhibited by the IL-1 receptor antagonist IL-1Ra.[1] A related system involves the three IL-36 cytokines IL-36α, IL-36β and IL-36γ, which are neutralized when the IL-36Ra binds the IL-36 receptor IL-1RL2 (also known as IL-36R). IL-36R is expressed on many epithelial, dendritic, monocyte and T cell subtypes, and all five agonists activate MAP kinases and NF-κB leading to increased expression of chemokines that promote recruitment of inflammatory cells.[1]

Interest in how the IL-36 system may contribute to skin diseases has grown dramatically in recent years. Conflicting data is reported in the literature regarding expression of the system in contact hypersensitivity. While some studies found increased expression of the three IL-36s,[2, 3, 4] this was not observed in other independent experiments.[5, 6] A recent investigation conducted in mice suggested that IL-36α might be involved in T cell priming in the lymph nodes during the development of contact hypersensitivity; however, IL-36α deficiency did not impact the disease phenotype in the skin as evaluated through ear thickness and gross histology.[7] Since some human studies observed upregulation of all three IL-36s[2, 3, 4] in affected skin and we previously demonstrated specific involvement of a single IL-36 in different mouse models of skin disease,[8] we here examined the possible involvement of each of the IL-36s and their antagonist IL-36Ra in the well-established DNFB (1-fluoro-2,4-dinitrobenzene) mouse model of contact hypersensitivity.

Address correspondence to: Liselotte E. Jensen, Ph.D., Department of Microbiology and Immunology, Temple University Lewis Katz School of Medicine, 1158 MERB, 3500 N. Broad Street, Philadelphia, PA 19140-4106, Phone: +215-707-8144, liselott@temple.edu.

CONFLICTS OF INTEREST

Ms. Zaladonis has nothing to disclose. Mr. Zhang has nothing to disclose. Dr. Manupipatpong has nothing to disclose. Dr. Kalaiselvan has nothing to disclose. Dr. Alvarez has nothing to disclose. Dr. Jensen reports grants from U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, during the conduct of the study.

All animal procedures were approved by the Temple University Lewis Katz School of Medicine Institutional Animal Care and Use Committee and performed in compliance with the U.S. Department of Health and Human Services Guide for the Care and Use of Laboratory Animals. The previously described[8] IL-36 cytokine knockout (KO) mice and controls were treated as described in figure 1A. Treatment with DNFB lead to similar ear swelling in all four strains (Fig. 1B, data pooled from 5 independent experiments and analyzed by ANOVA). Although male ears were significantly ($p<0.01$) heavier than female ears, no statistically significant differences between mouse strains were identified. In agreement with this, histological analyses of epidermal and dermal thickness did not reveal statistically significant differences between wild type, and IL-36α, IL-36β or IL-36γ deficient mice (Fig. 1C–D). Similar preliminary analyses of shorter sensitization (4 days) or challenge periods (6 and 18 hours) did not reveal strain dependent differences either. While ear thickness is an outcome commonly used to determine disease severity, the approach has its limitations. Hence, we also evaluated expression levels of a number of cytokines linked to the disease phenotype[6] and previously shown to be regulated by IL-36 directly or indirectly.[1] Inflammation was clearly induced in all four strains, as chemokines not measurable in normal ears were readily detected after DNFB treatment. However, in agreement with the gross ear swelling data (Fig. 1B–D), no statistically significant differences could be detected between mouse strains (Fig. 1E).

The balance between pro-inflammatory agonists to anti-inflammatory antagonists is naturally important for the final outcome in many situations. In allergic contact dermatitis patients expression of IL-1β, IL-1Ra, IL-36α, IL-36β and IL-36γ is reported to be higher in involved skin than uninvolved sites.[2] Interestingly, expression of IL-36Ra appears unchanged, thus, prompting the hypothesis that low IL-36Ra expression could be part of the pathogenesis.[2] Consequently, we next examined the effect of IL-36Ra deficiency *in vivo* in the DNFB mouse model (Fig. 2). If IL-36Ra plays an important role in limiting contact hypersensitivity in the model used, we would expect more severe disease in the IL-36Ra KO mice compared to wild type controls; however, all parameters measured in the model (ear weight (Fig. 2A), epidermal and dermal thickness (Fig. 2B–C) and cytokine expression (Fig. 2D)) were found to be similar to those in wild type mice. Hence, it appears that IL-36Ra is not an essential regulator of the immune response in at least the here used model at the examined timepoints.

A recent contact hypersensitivity study conducted in mice found an effect of deleting IL-36α when T cell priming was examined *ex vivo*; however, there was no effect upon disease outcome *in vivo*.[7] We here confirm that IL-36α deficiency does not impact severity of the DNFB induced inflammation (Fig. 1) and further establish that deletion of IL-36β or IL-36γ also does not impact the phenotype (Fig. 1). It remains a possibility that the three IL-36 cytokines act together to amplify priming and/or inflammatory responses; further analyses in IL-36R KO mice could test this hypothesis. However, such a combined function cannot be regulated by IL-36Ra as we did not observe an effect of deleting IL-36Ra (Fig. 2).

The previous study of hypersensitivity in IL-36α KO mice found no increase in IL-36α expression in the challenged inflamed skin, and this lack of induction was proposed as a possible explanation for the lack of an *in vivo* phenotype.[7] In this context, it is noteworthy

that while some human studies have observed increased expression of the IL-36 cytokines in affected skin compared to uninvolved sites,[2, 3, 4] others did not identify such changes.[5, 6] Unlike the laboratory mice which are genetically identical and are challenged with a single agent in each experiment, human patients are genetically diverse and develop allergies to a wide range of allergens. The present (Figs. 1–2) and previous[7] study of hypersensitivity in mice employed the DNFB model in C57BL/6 mice, and found no evidence for involvement of IL-36 or IL-36Ra in induction of acute disease as determined 24 hours after challenge. However, it remains a possibility that different allergens and/or genetic backgrounds could reveal a role for the IL-36 system in initiation of allergies. Interestingly, a more recent study revealed a role for IL-36Ra 48 hours post-challenge,[9] which suggests involvement in chronic disease. Hence, future studies should be designed accordingly.

ACKNOWLEDGEMENT

This research was supported by R01 grant AI125111 to LEJ from The National Institute of Allergy and Infectious Diseases. The Histopathology Facility at Fox Chase Cancer Center, where tissues were processed for histology, is supported by the core grant P30 CA006927 from The National Cancer Institute.

REFERENCES

1. Jensen LE. Interleukin-36 cytokines may overcome microbial immune evasion strategies that inhibit interleukin-1 family signaling. Science Signaling 2017;10(492):eaan3589. [PubMed: 28811383]
2. Mattii M, Ayala F, Balato N, Filotico R, Lembo S, Schiattarella M, Patruno C, Marone G, Balato A. The balance between pro- and anti-inflammatory cytokines is crucial in human allergic contact dermatitis pathogenesis: the role of IL-1 family members. Exp Dermatol 2013;22(12):813–819. [PubMed: 24164463]
3. Lembo S, Caiazzo G, Balato N, Monfrecola G, Patra V, Wolf P, Balato A. Polymorphic light eruption and IL-1 family members: any difference with allergic contact dermatitis? Photochemical & Photobiological Sciences 2017;16(9):1471–1479. [PubMed: 28812775]
4. Balato A, Mattii M, Caiazzo G, Raimondo A, Patruno C, Balato N, Ayala F, Lembo S. IL-36γ Is Involved in Psoriasis and Allergic Contact Dermatitis. J Invest Dermatol 2016;136(7):1520–1523. [PubMed: 27021407]
5. D'Erme AM, Wilsmann-Theis D, Wagenpfeil J, Holzel M, Ferring-Schmitt S, Sternberg S, Wittmann M, Peters B, Bosio A, Bieber T, Wenzel J. IL-36γ (IL-1F9) is a biomarker for psoriasis skin lesions. J Invest Dermatol 2015;135(4):1025–1032. [PubMed: 25525775]
6. Quaranta M, Knapp B, Garzorz N, Mattii M, Pullabhatla V, Pennino D, Andres C, Traidl-Hoffmann C, Cavani A, Theis FJ, Ring J, Schmidt-Weber CB, Eyerich S, Eyerich K. Intraindividual genome expression analysis reveals a specific molecular signature of psoriasis and eczema. Science Translational Medicine 2014;6(244):244ra290.
7. Numata T, Yoshizaki T, Yamaguchi S, Shimura E, Iwakura Y, Harada K, Sudo K, Tsuboi R, Nakae S. IL-36α is involved in hapten-specific T-cell induction, but not local inflammation, during contact hypersensitivity. Biochem Biophys Res Commun 2018;506(3):429–436. [PubMed: 30352688]
8. Milora KA, Uppalapati SR, Sanmiguel JC, Zou W, Jensen LE. Interleukin-36β provides protection against HSV-1 infection, but does not modulate initiation of adaptive immune responses. Sci Rep 2017;7(1):5799. [PubMed: 28724920]
9. Fukushima H, Iwata Y, Watanabe S, Saito K, Tanaka Y, Hasegawa Y, Akiyama M, Sugiura K. TAK-242 ameliorates contact dermatitis exacerbated by IL-36 receptor antagonist deficiency. Sci Rep 2020;10(1):734. [PubMed: 31959814]

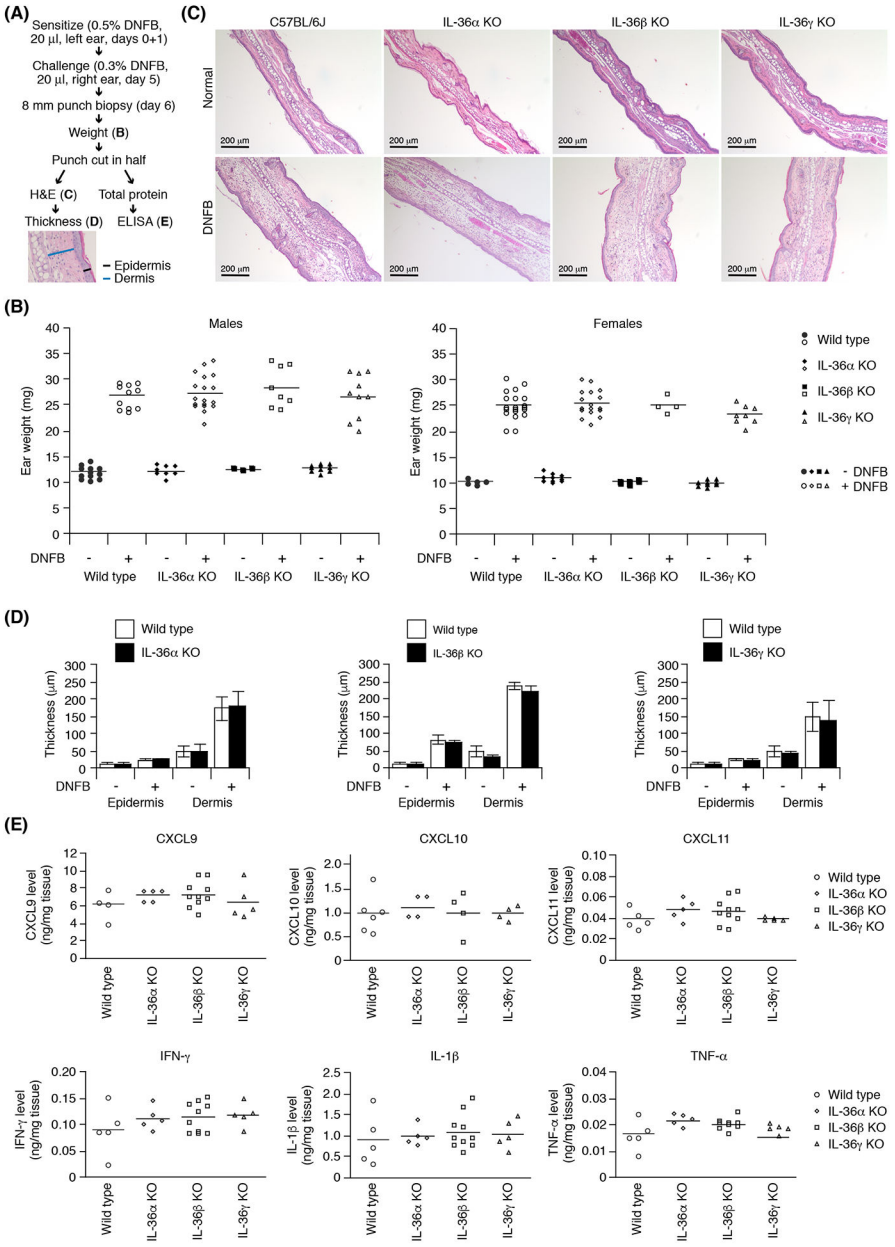

FIGURE 1.

IL-36 deficiency does not impact acute DNFB induced hypersensitivity. Mice were treated with DNFB (Sigma-Aldrich) in acetone/olive oil (3:1 vol/vol) and evaluated as outlined (A). Standardized ear samples were collected using punch biopsy tools. Tissue weight was determined (B) before the sample was cut in half. One half was used for histology (C) and evaluation of epidermal and dermal thickness (D); the other for ELISAs (R&D Systems, E). Thickness was determined using ImageJ software by drawing straight lines through the epidermis and dermis (A). Three sites were measured and averaged per tissue/mouse. No statistically significant differences between strains were identified.

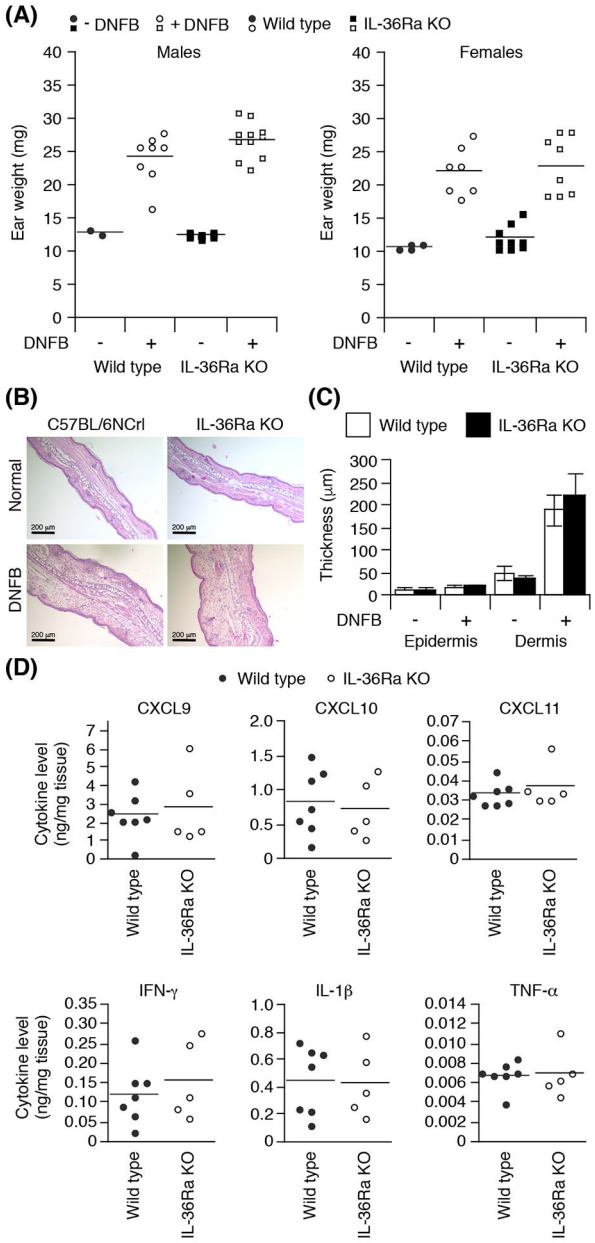

FIGURE 2.

Acute DNFB induced contact hypersensitivity is not aggravated in the absence of IL-36Ra. Mice deficient in IL-36Ra were obtained from the Mutant Mouse Resource & Research Centers (strain B6N.129S5-*Il1f5*^{tm1Lex}/Mmucd) and genotyped using primers Neo3a(Il1f5) GCAGCGCATCGCCTTCTATC, UNQ1896–16 GAGCTCCATGATGTTCACTGG, UNQ1896–7 GAGCTGCATCCTGTCATCG and UNQ1896–8 CCTCTAGCTGATATACATCC. IL-36Ra KO and C57BL/6NCrl background matched wild type mice were treated and analyzed as described in figure 1. Tissue weight (A), histology (H&E staining, B), epidermal and dermal thickness (C) and cytokine levels (E) were evaluated. No statistically significant differences between strains were identified.